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**MONOCLONAL ANTIBODIES DIRECTED AGAINST SURFACE MOLECULES
OF MULTICELL SPHEROIDS**

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Introduction

The objective of this project is to generate a library of monoclonal antibodies (MAbs) directed against surface molecules of tumor and transformed cells grown as multicell spheroids (MCS). These MCS are highly organized, 3-dimensional multicellular structures which exhibit many characteristics of *in vivo* organized tissues not found in conventional monolayer or suspension culture. Therefore MCS make better *in vitro* model systems to study the interactions of mammalian cells, and provide a functional assay for surface adhesion molecules.

This project also involves investigations of cell-cell interactions in a gravity-based environment. It will provide a base of scientific information necessary to expand the focus of the project in future years to microgravity and hypergravity-based environments. This project also has the potential to yield important materials (e.g., cellular products) which may prove useful in the diagnosis and/or treatment of certain human diseases. Moreover, this project supports the training of both undergraduate and graduate students; thus, it will assist in developing a pool of future scientists with research experience in an area (gravitational biology) of interest to NASA.

Research Progress Summary

The aims for this reporting period were: (1) to continue to expand our MAb library; (2) to continue to characterize the binding pattern and binding intensity of selected MAbs on panels of human and rodent cell lines; and (3) to initiate functional biological assays to determine whether MAbs can modulate the cell-cell interactions (i.e., aggregation, compaction) of normal, tumor, or transformed cells.

Additional fusions resulting in a variety of MAbs directed against a human neuroblastoma cell line were performed and hybridomas are currently being cloned. In addition, three new MAbs directed against WI38SV40 fibroblasts were cloned (WSJ-7 through WSJ-9). These MAbs exhibit strong binding to human cells and slight binding to rodent cells. The binding patterns of these MAbs are currently being analyzed further by flow cytometry.

MAbs identified in the last report were characterized by immunofluorescence microscopy and flow cytometry on panels of human and rodent cells. All MAbs (WSJ-2 through WSJ-6) directed against WI38SV40 cells exhibited strong binding to transformed fibroblasts and slight binding to normal (diploid) fibroblasts. Further, MAbs which exhibiting a "cell surface" binding pattern exhibited much stronger binding to HeLa (human cervical carcinoma) cells. It is also interesting to note that two MAbs also exhibited slight binding to CHO 77256 (Chinese Hamster Ovary) cells but not to other rodent cells. Six MAbs directed against B14I50 wildtype and 2DF*F1 mutant hamster cells reported previously were characterized further by immunofluorescence and flow cytometry on panels of human and rodent cells. Five MAbs reacted specifically with rodent cells while one MAb (BSG1.1) reacted

with both human and rodent cells

Preliminary aggregation and compaction functional studies were carried out on 96-well tissue culture plates coated with 0.75% agarose solution and seeded with 10^3 to 10^4 cells/well. Fab fragments of one MAb, MTS 1.2, developed against MTS of WI38SV40 lung fibroblasts, but not whole antibody, appeared to block cell aggregation. The assay will be repeated with appropriate controls to confirm these results. A sample of bovine serum albumin (or other appropriate protein) will be run through the Fab fragment procedure to ensure that the results are not due to non-specific cytotoxic effects. Additionally, a MAb directed against a non-WI38SV40 surface antigen will be used to exclude the possibility that the blocking of aggregation is due to high antibody concentration.

The functional biological assay was also applied to recent fusions. Supernatants from wells of 96-well plates containing hybridomas were tested to see if the supernatants modulated cell aggregation and/or compaction. This will be a useful assay to screen all hybridoma supernatants as opposed to only screening selected MAbs. First screenings of two recent fusions resulted in the identification of number of supernatants which appeared to block aggregation. These screenings are currently being repeated to confirm the preliminary results. Various other aspects of the functional assays, such as utilizing a shaker platform to ensure uniform distribution of cells in the wells during incubation, and searching for a commercial antibody which can be used as a positive control for the assays are also being pursued.

This project also supported the training of two underrepresented minority students (one graduate and one undergraduate) during the reporting period. Both students made significant progress in their research training. Moreover, each student submitted an abstract and presented a paper at a national scientific meeting. One student (Cynthia Cantu) completed requirements for the M.S. degree in biology and was accepted to the University of Texas Medical School at Houston. She will start in the fall.

Future Plans

The specific aims for the next six-month period are: (1) to continue the cloning, expansion, characterization of MAbs; and (2) to continue functional assays and initiate Western blot analysis of surface molecules with selected MAbs from our library.

Appendix

RESEARCH
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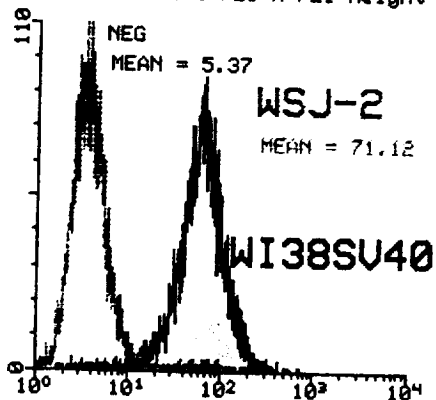
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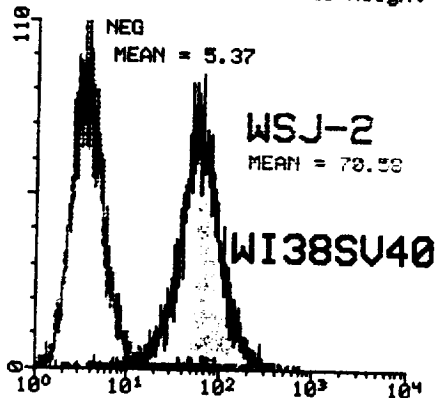
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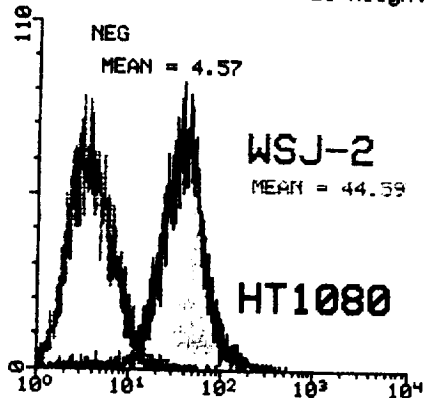


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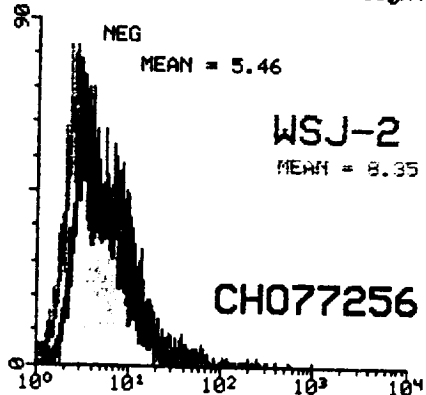


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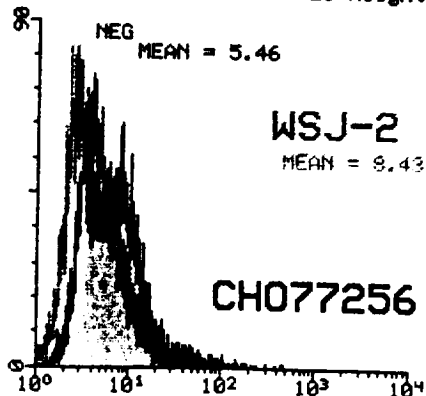
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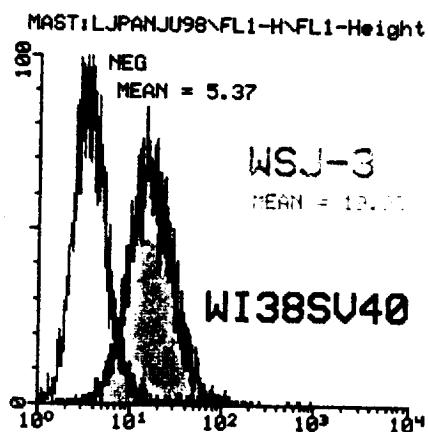
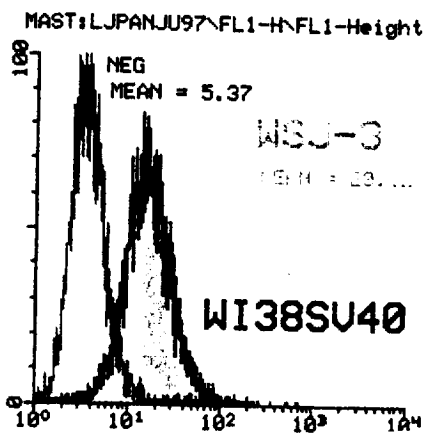
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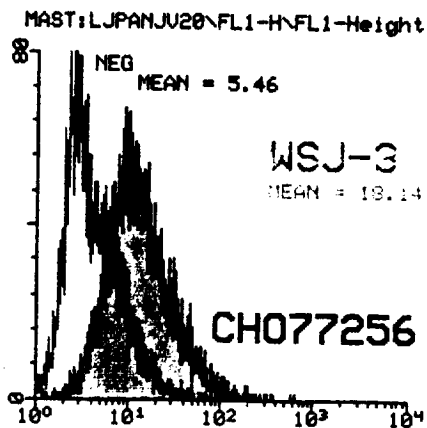
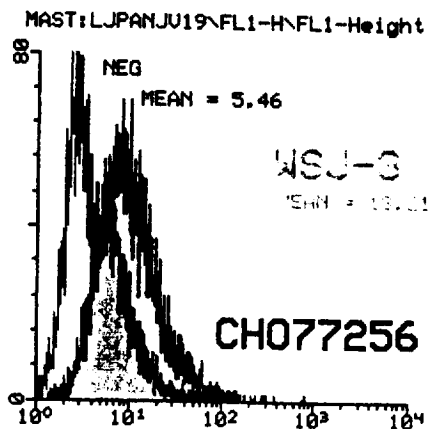
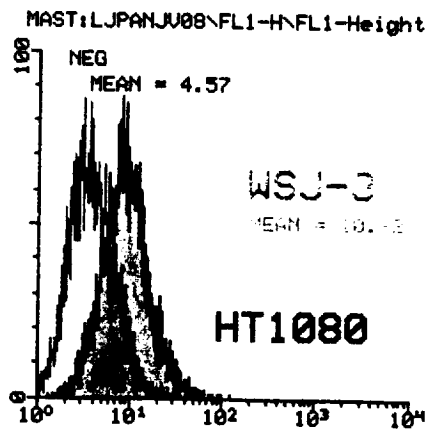
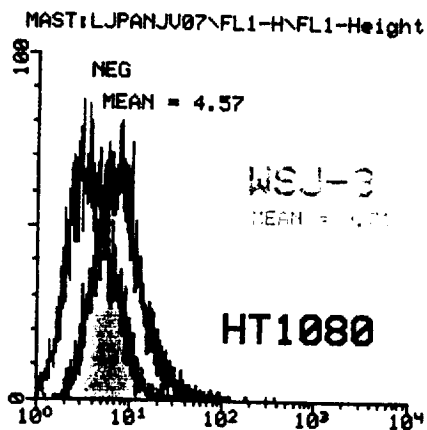
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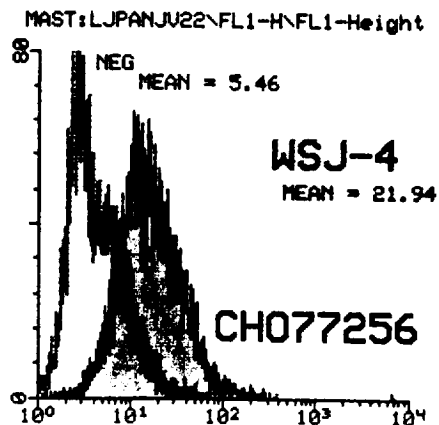
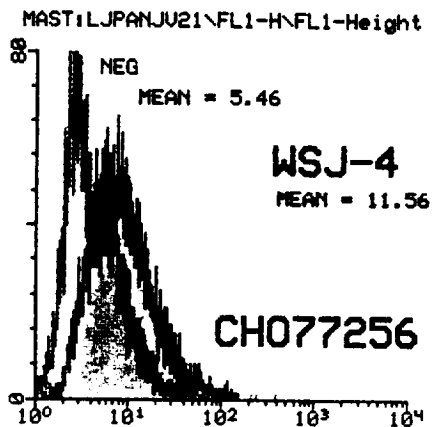
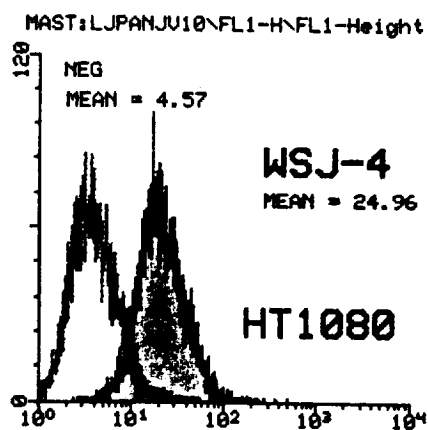
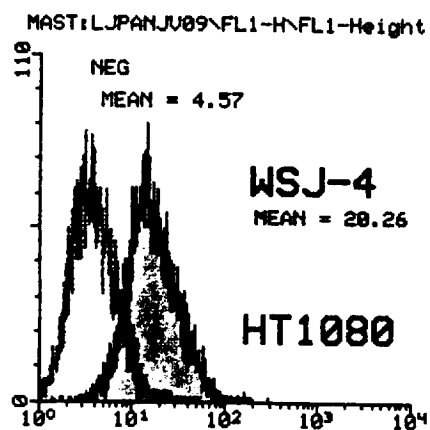
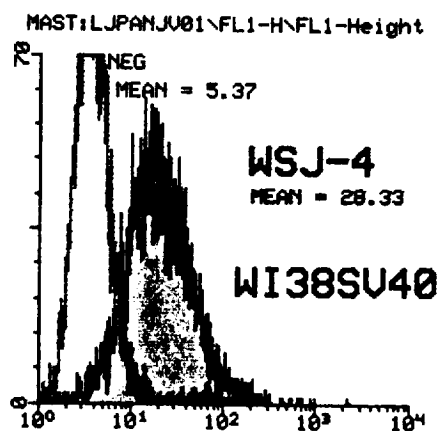
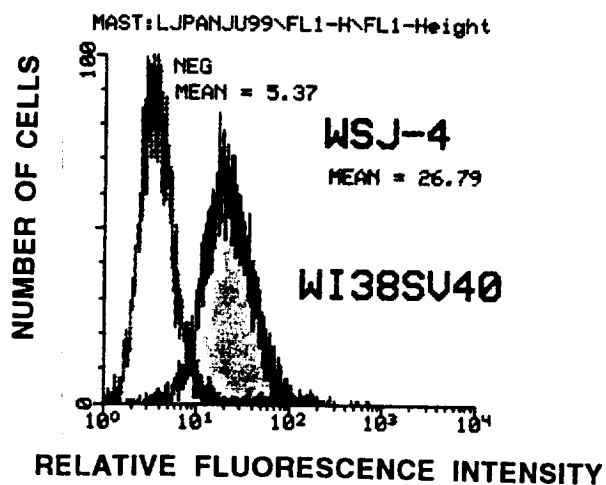


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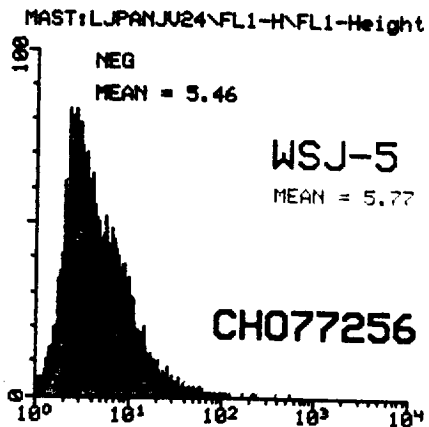
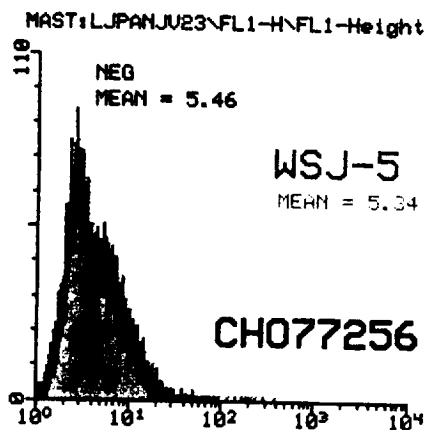
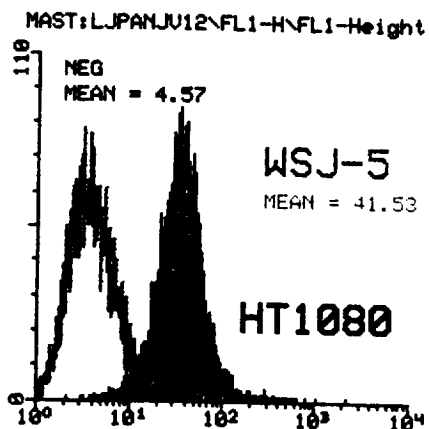
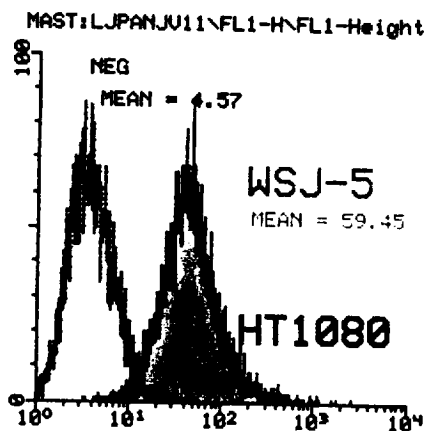
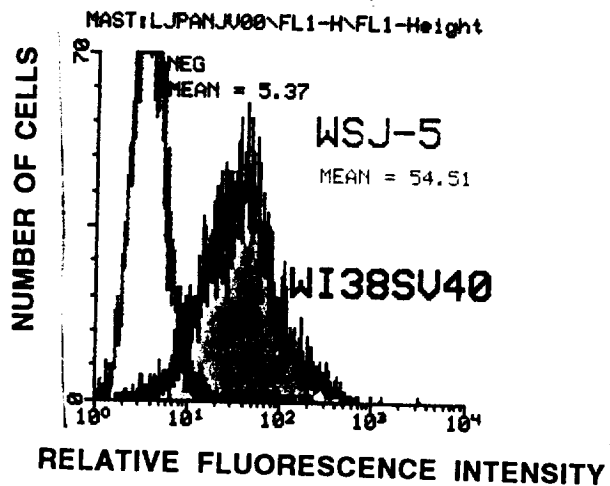


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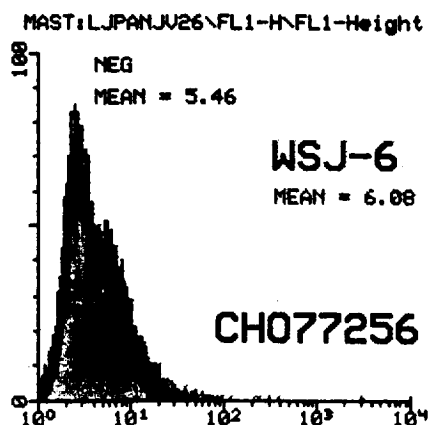
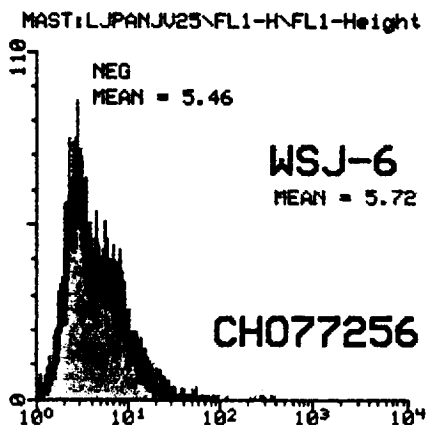
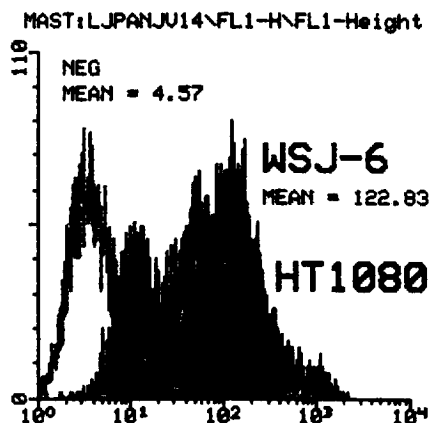
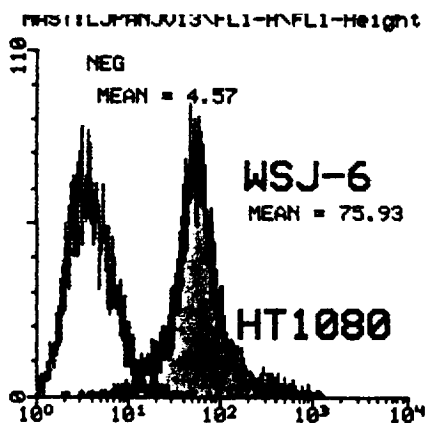
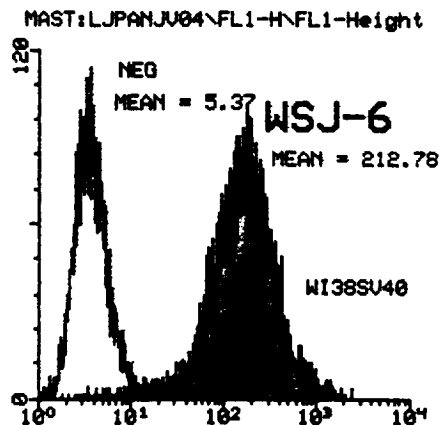
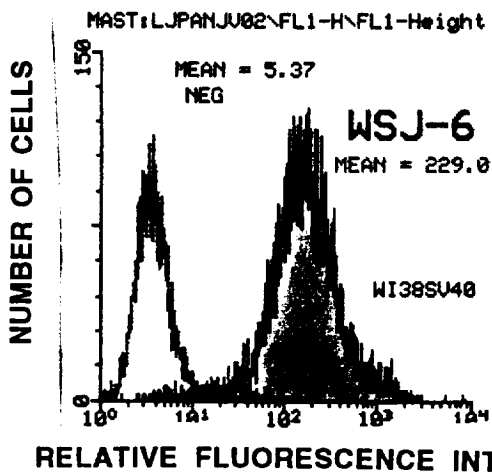


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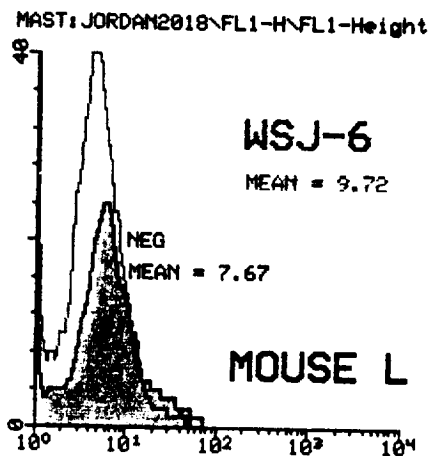
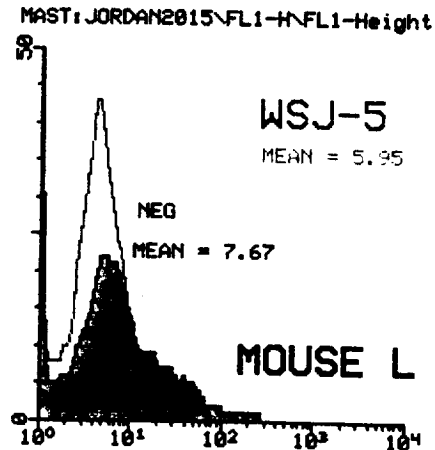
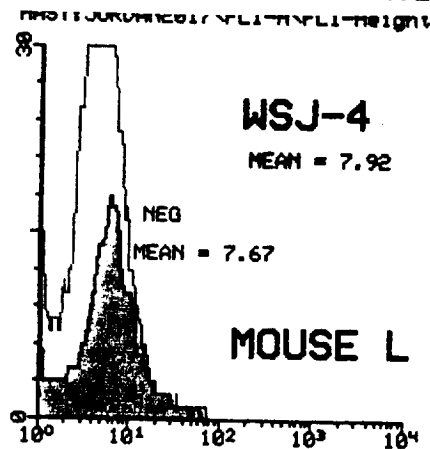
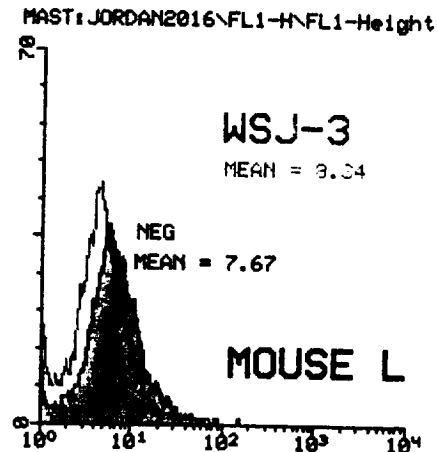
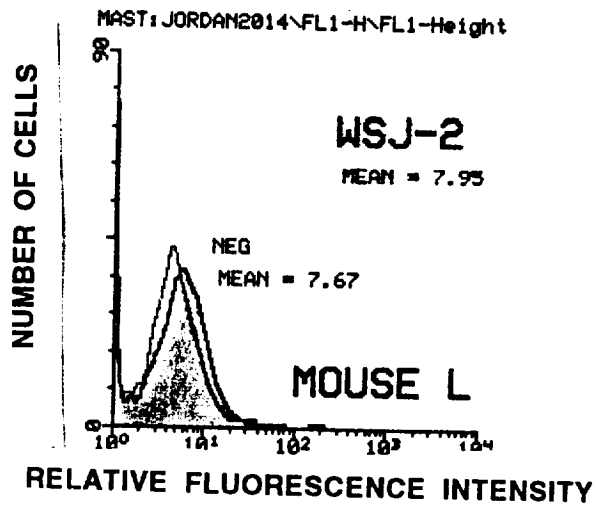


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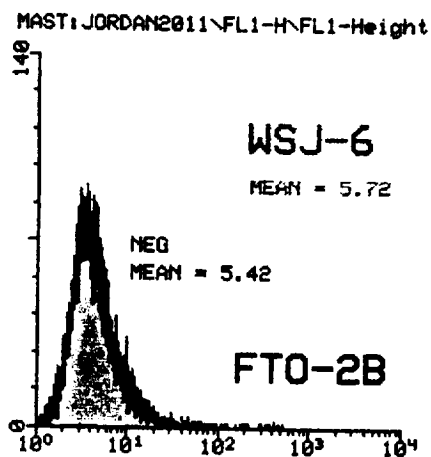
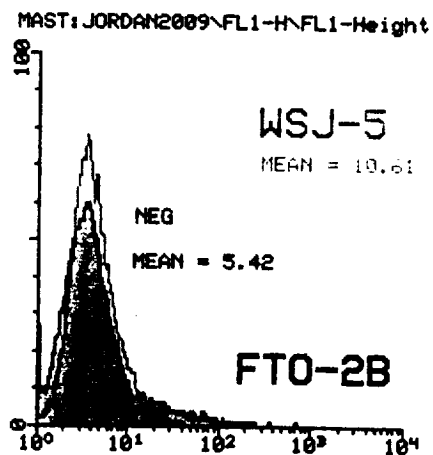
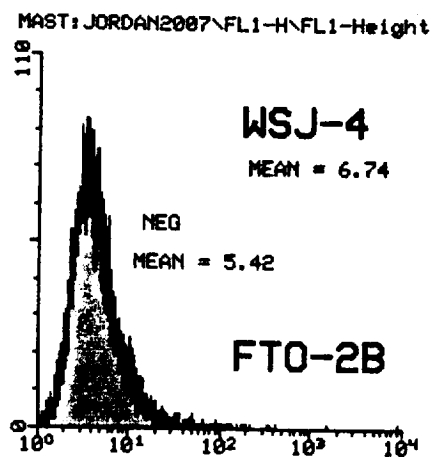
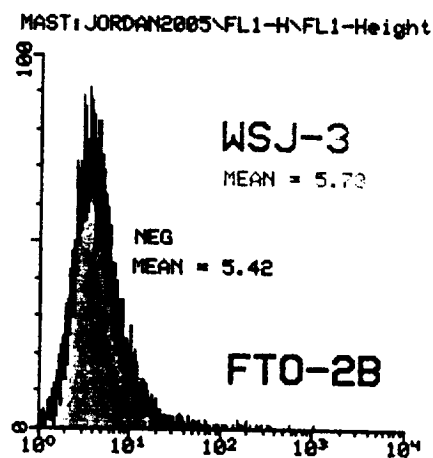
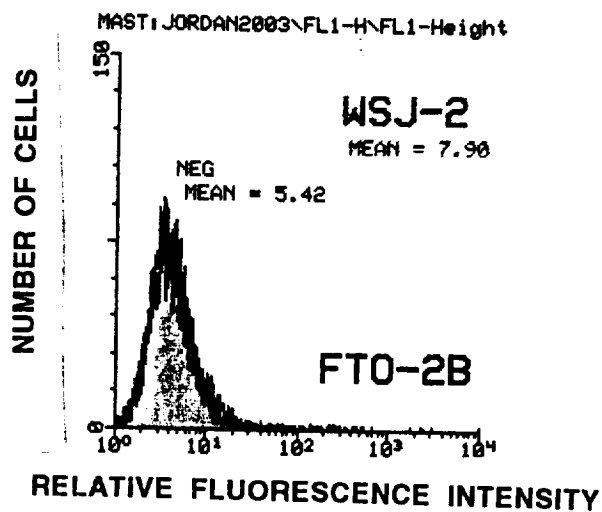


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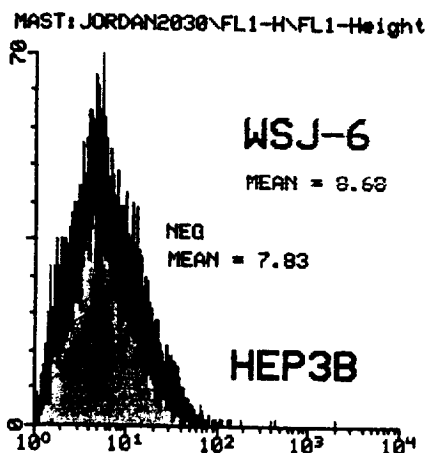
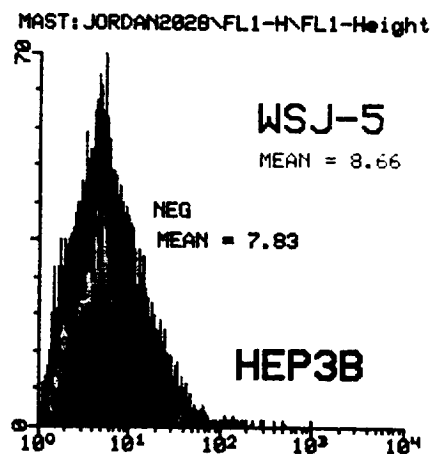
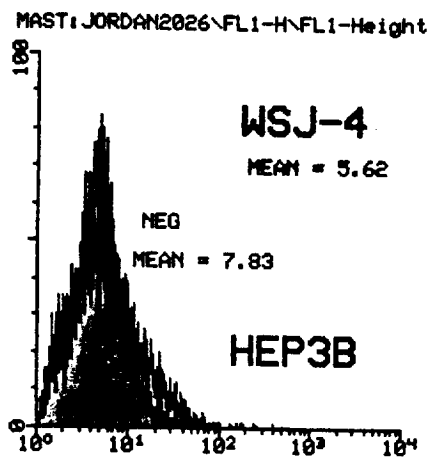
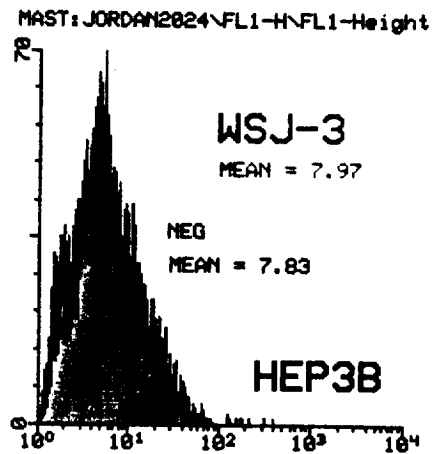
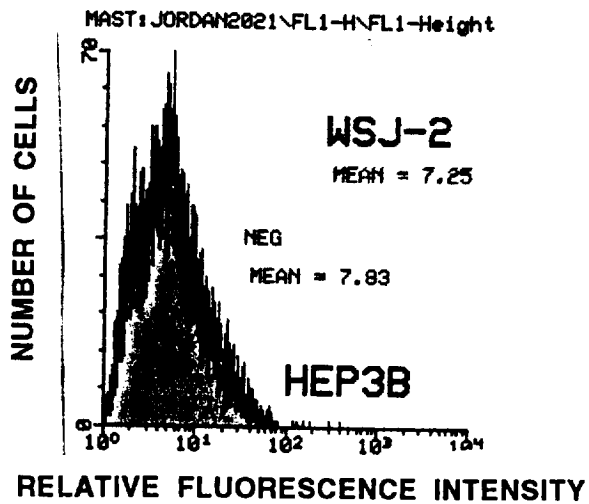


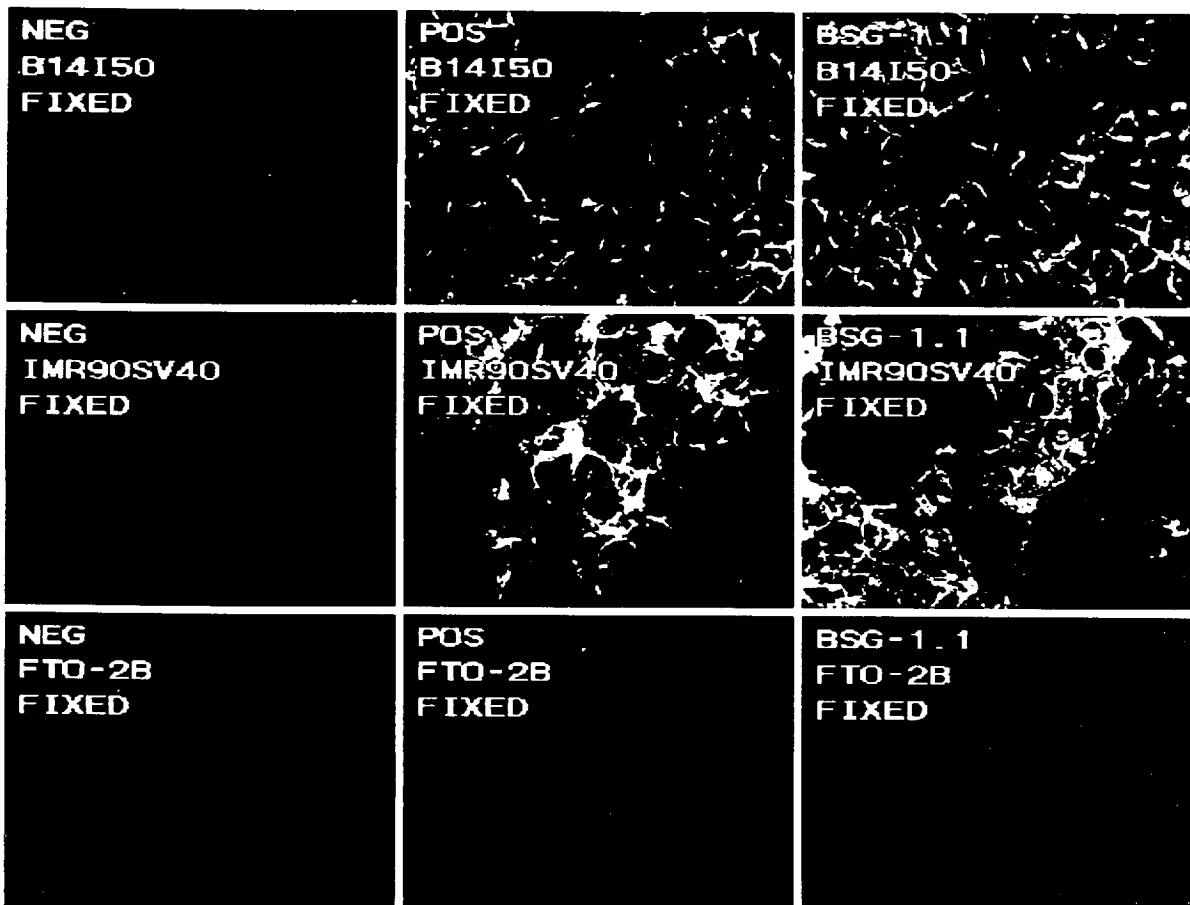
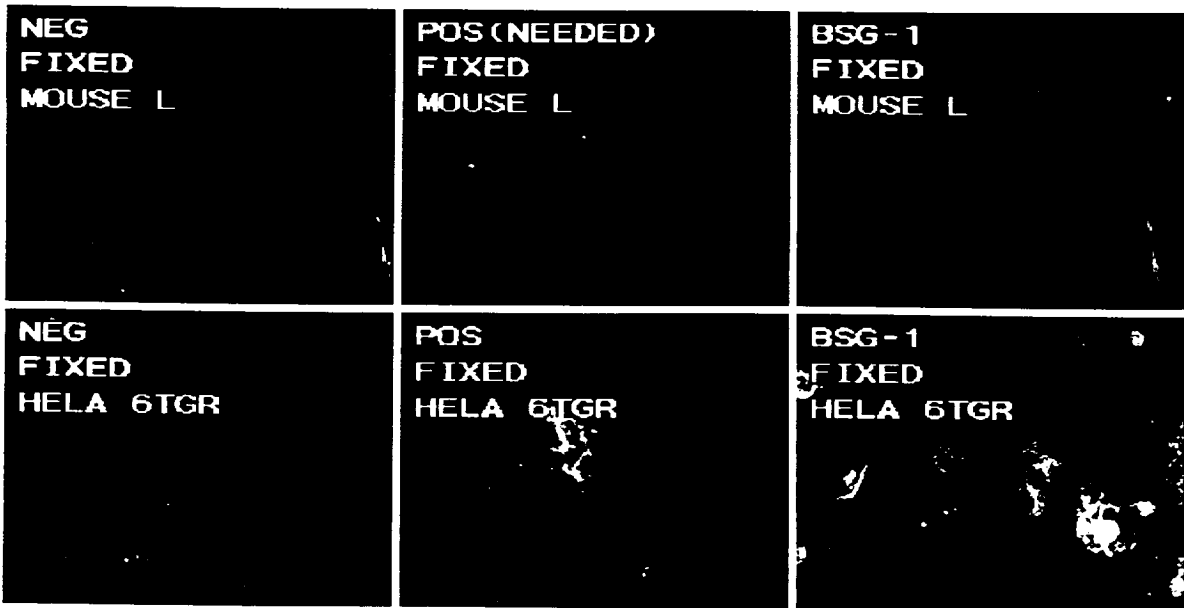
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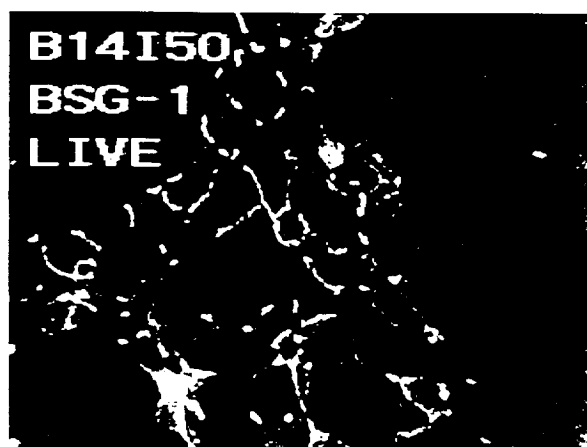
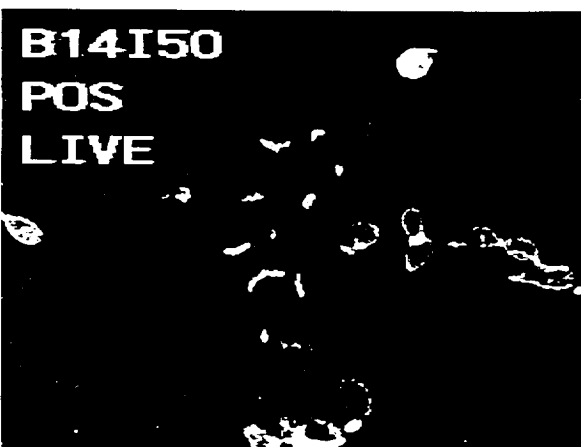
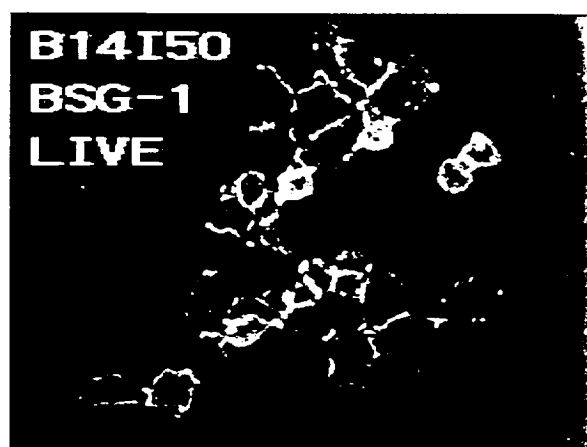
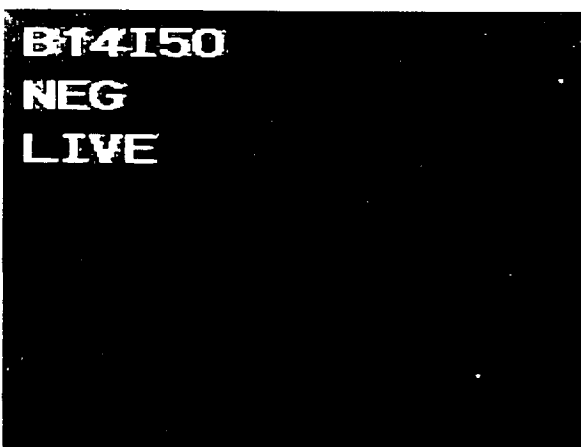
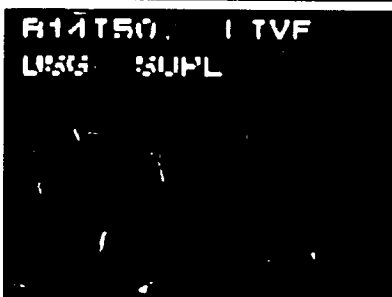
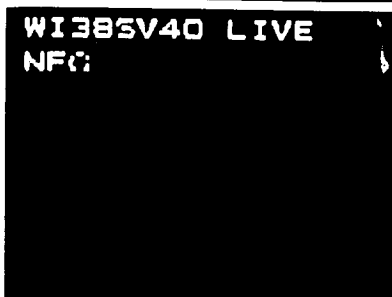
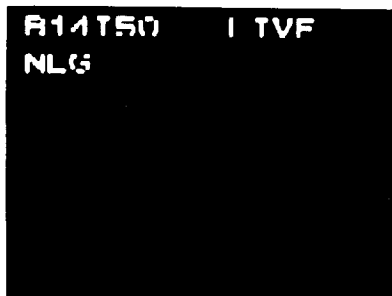
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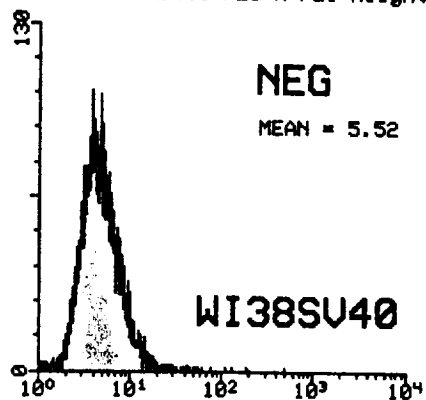
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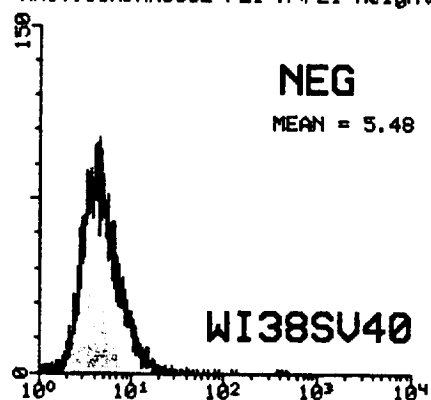
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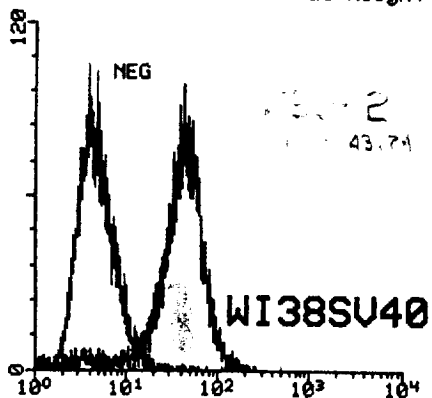
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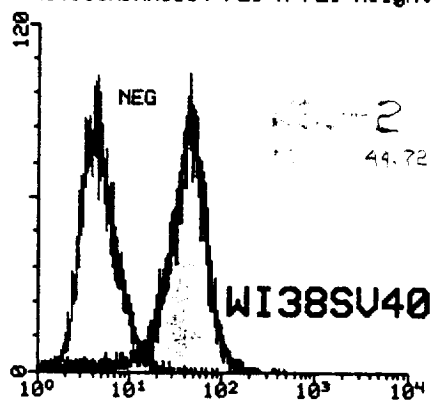
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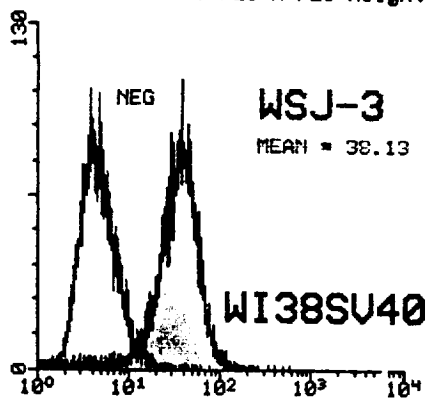
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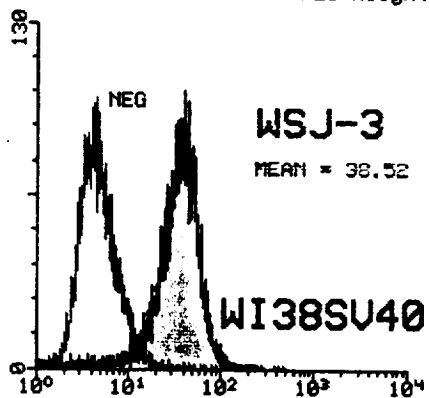
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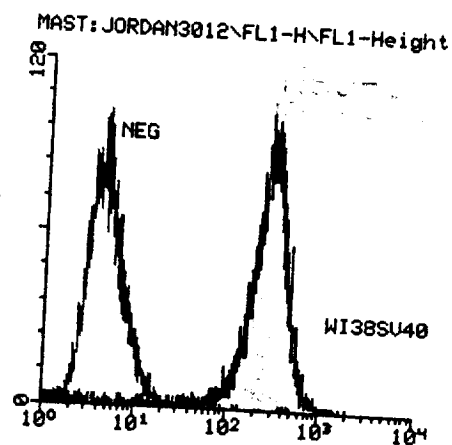
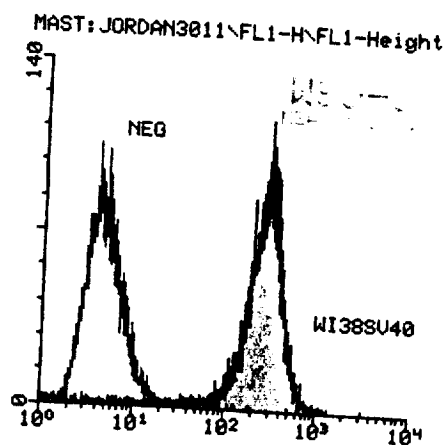
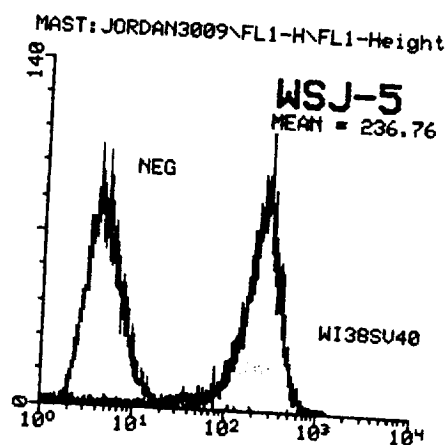
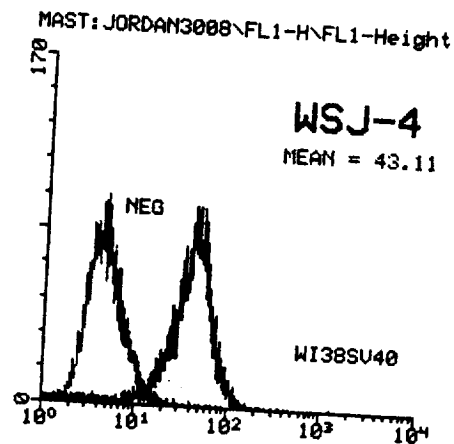
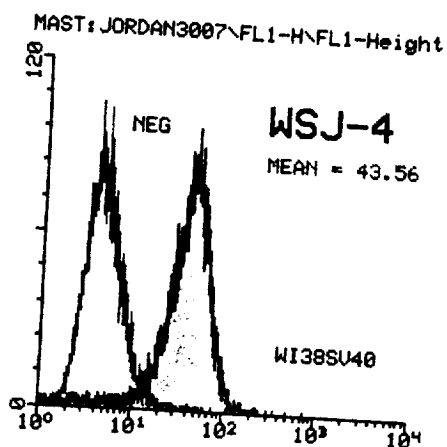


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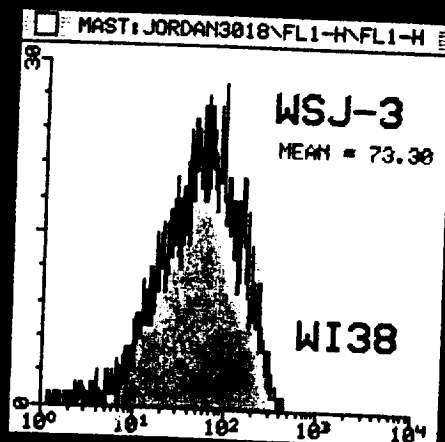
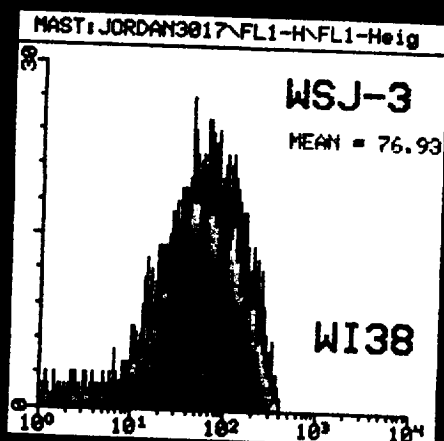
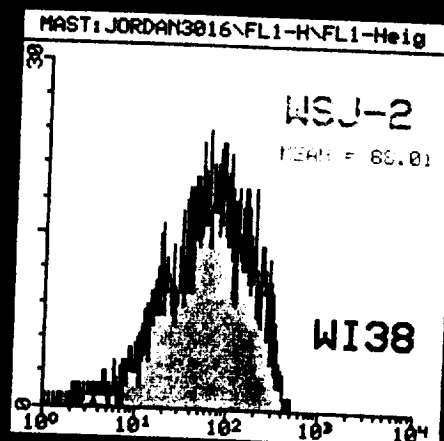
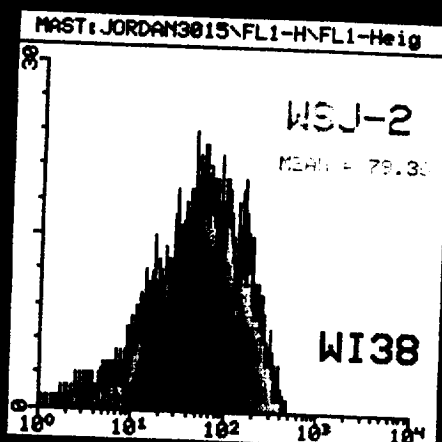
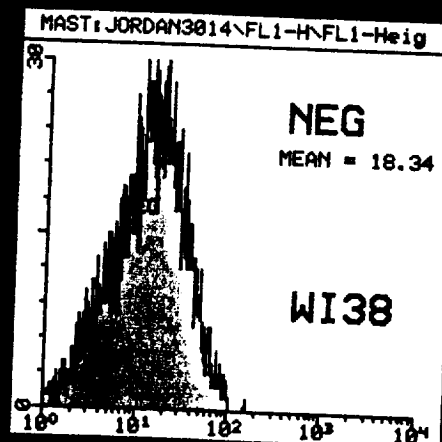
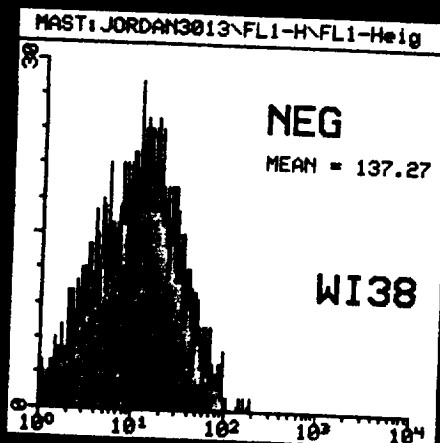


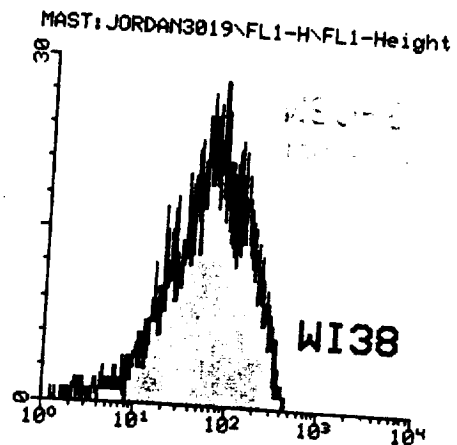
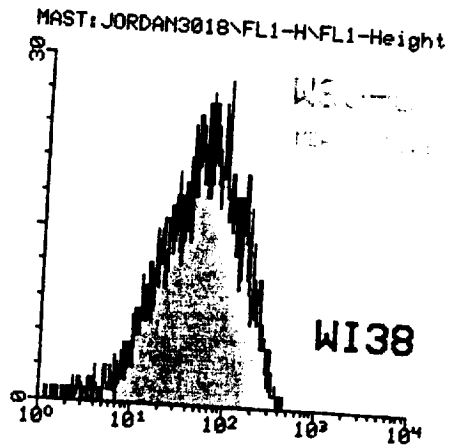
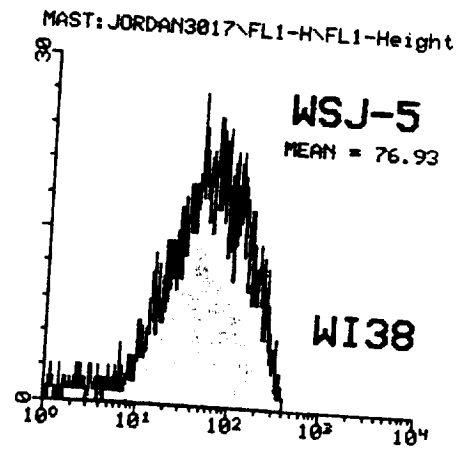
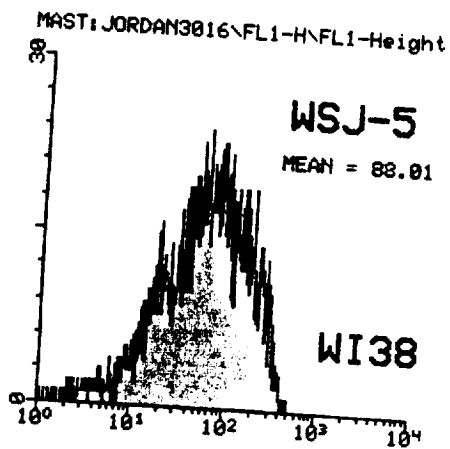
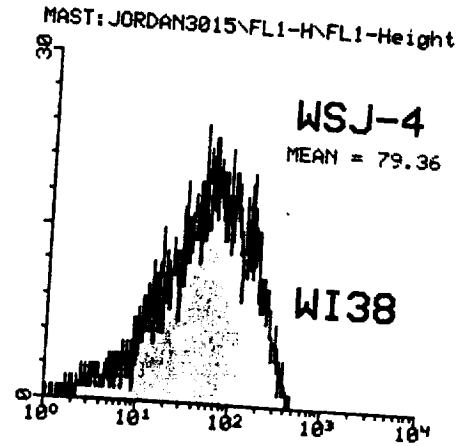
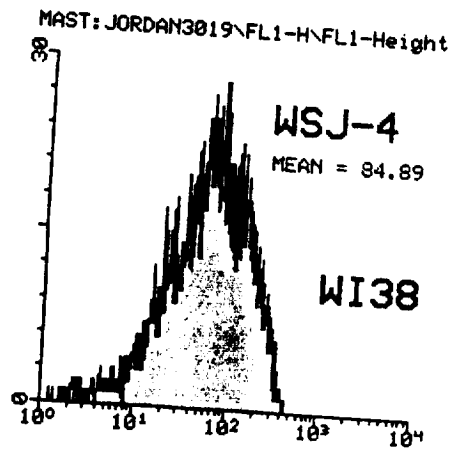
MAST: JORDAN3006\FL1-H\FL1-Height

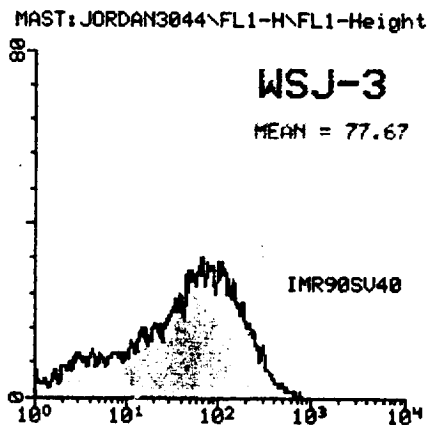
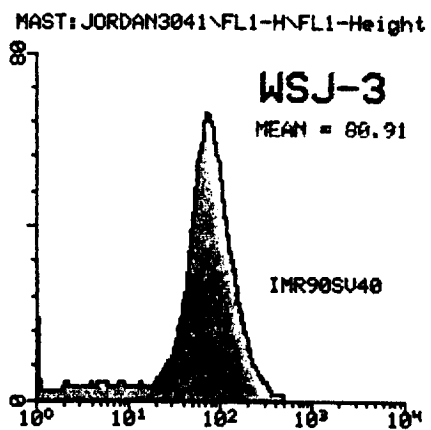
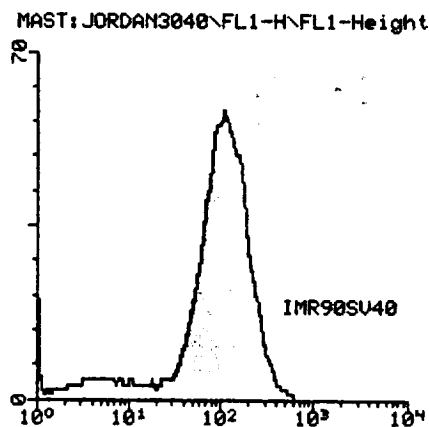
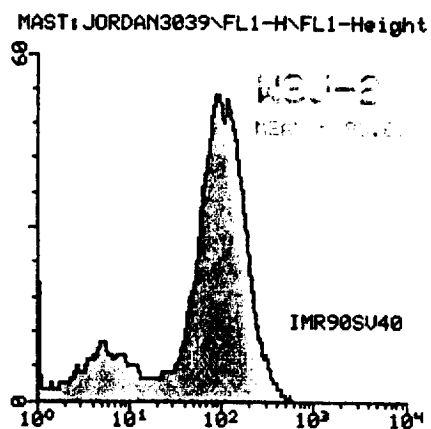
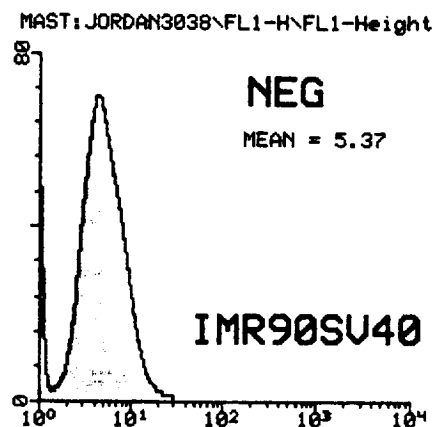
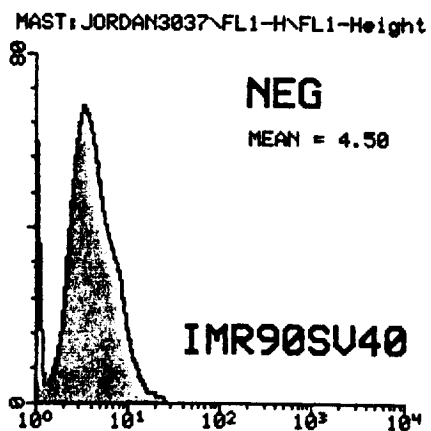


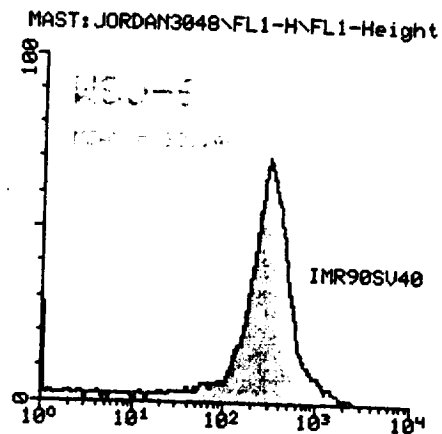
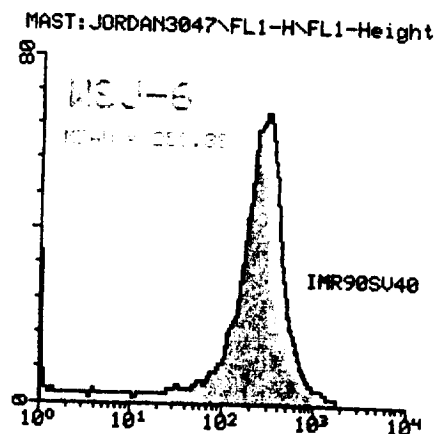
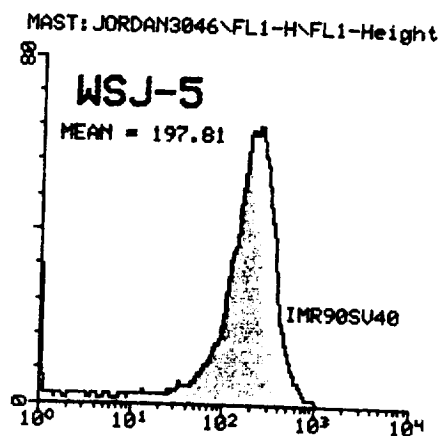
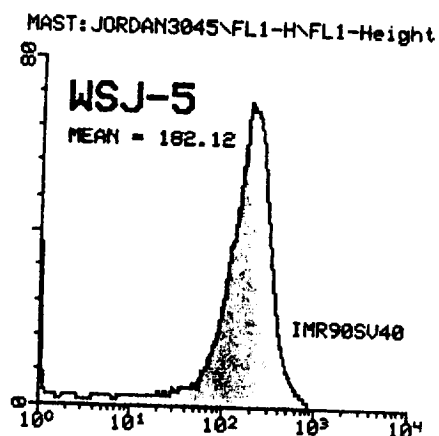
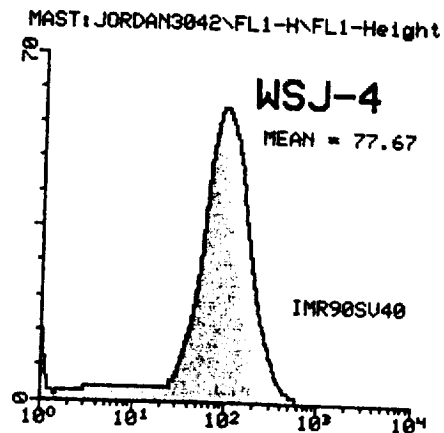
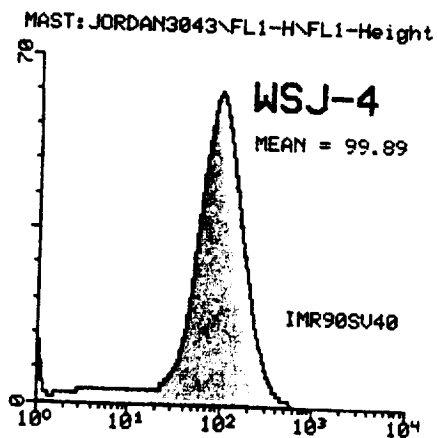


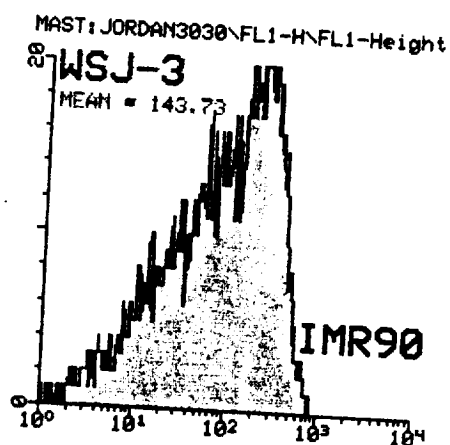
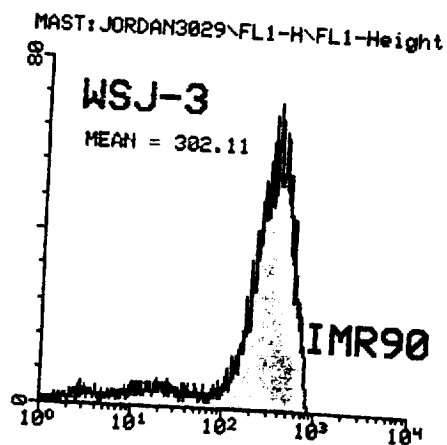
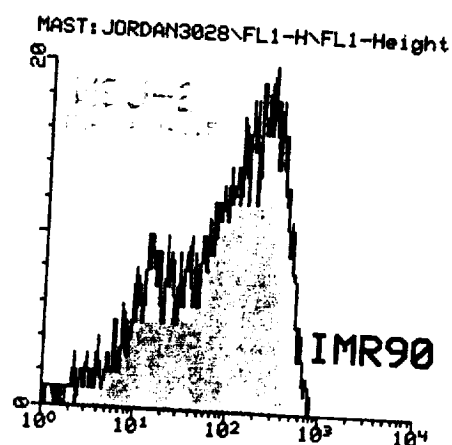
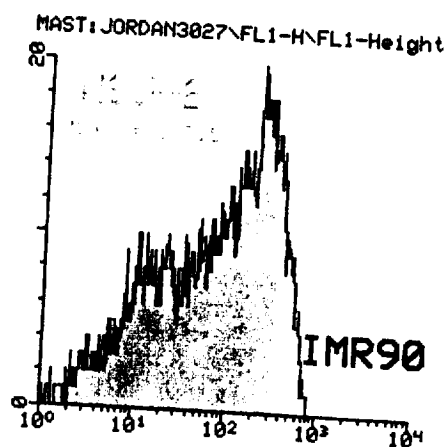
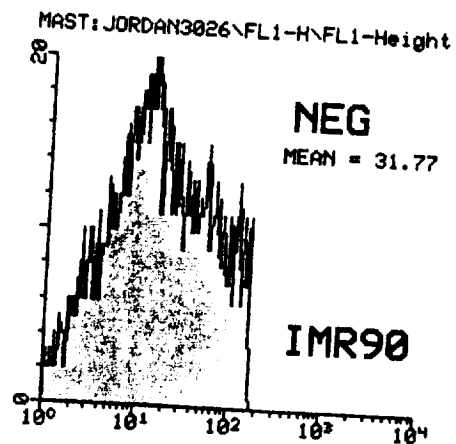
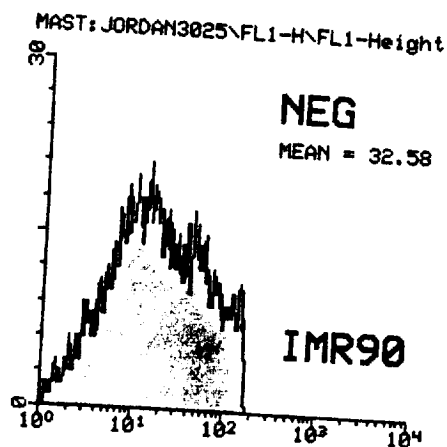
LYS CMD HIST HSTATS DOT-PLOT CONTOUR/3D 2D-STATS GATES WINDOWS



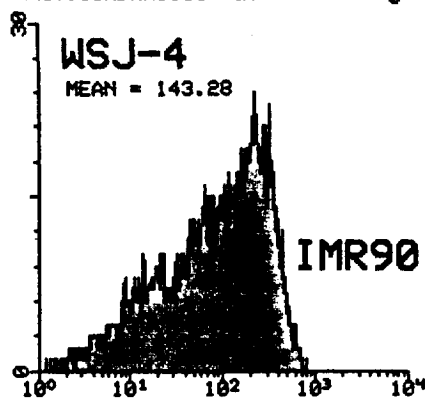




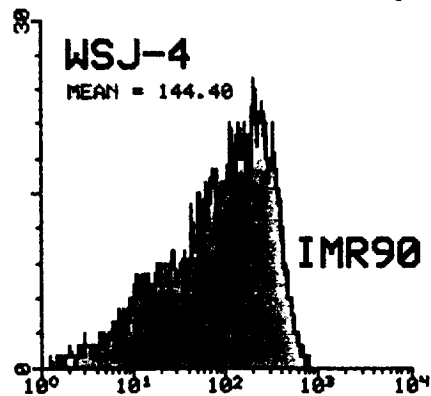




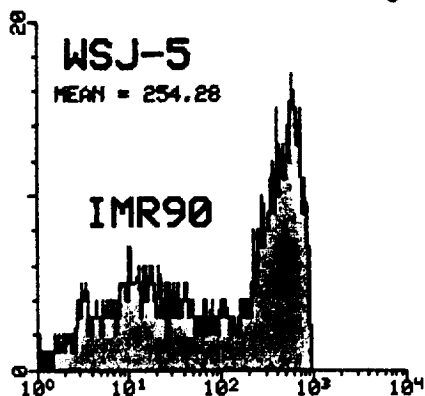
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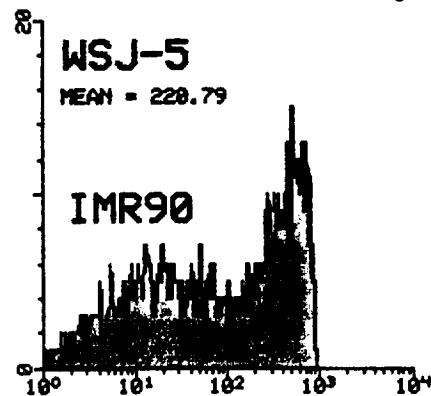
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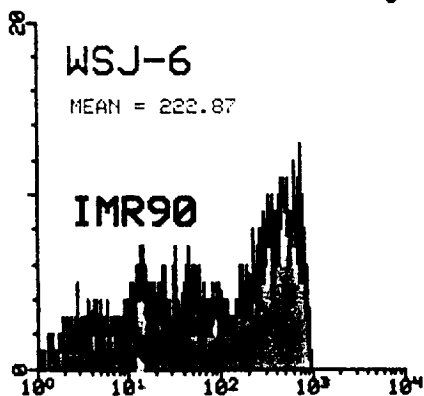
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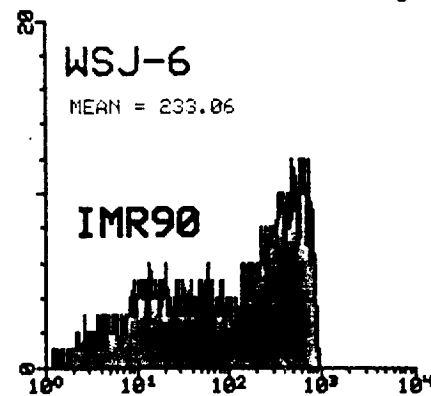
MAST: JORDAN3034\FL1-H\FL1-Height



MAST: JORDAN3035\FL1-H\FL1-Height



MAST: JORDAN3036\FL1-H\FL1-Height



FLOW CYTOMETRY SUMMARY

CELL LINE	ANTIBODY	FLOW CYTOMETRY	
		<u>*NET FLUORESCENCE</u>	
WI38sv40	WSJ-2	+++	(333.27)
	WSJ-3	+++	(308.67)
	WSJ-4	+++	(316.99)
	WSJ-5	+++	(367.87)
	WSJ-6	++++	(413.83)
WI38	WSJ-2	++	(236.02)
	WSJ-3	++	(210.45)
	WSJ-4	++	(219.70)
	WSJ-5	+++	(390.22)
	WSJ-6	++++	(423.81)
IMR90	WSJ-2	+	(169.7)
	WSJ-3	+	(166.4)
	WSJ-4	+	(166.5)
	WSJ-5	++	(222.3)
	WSJ-6	++	(217.7)
IMR90sv40	WSJ-2	+++	(338.1)
	WSJ-3	+++	(308.7)
	WSJ-4	+++	(321.3)
	WSJ-5	++++	(405.8)
	WSJ-6	++++	(450.7)
Hela 6TGr-	WSJ-2	+	(173.41)
	WSJ-3	+	(166.44)
	WSJ-4	+	(165.66)
	WSJ-5	++++	(401.68)
	WSJ-6	++++	(452.11)

FLOW CYTOMETRY SUMMARY CONT.

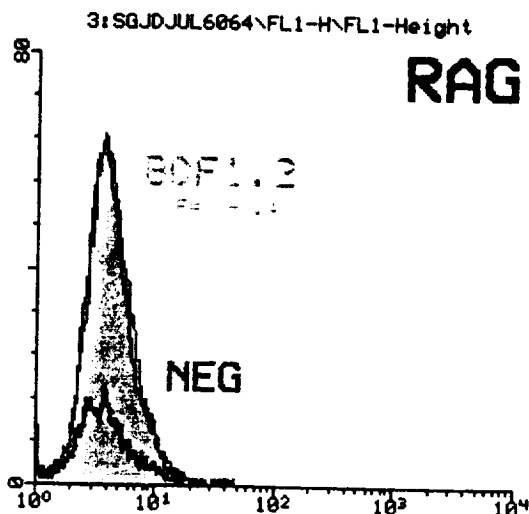
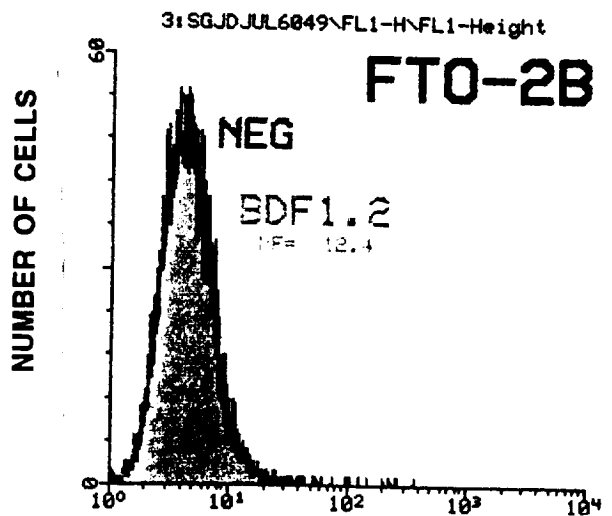
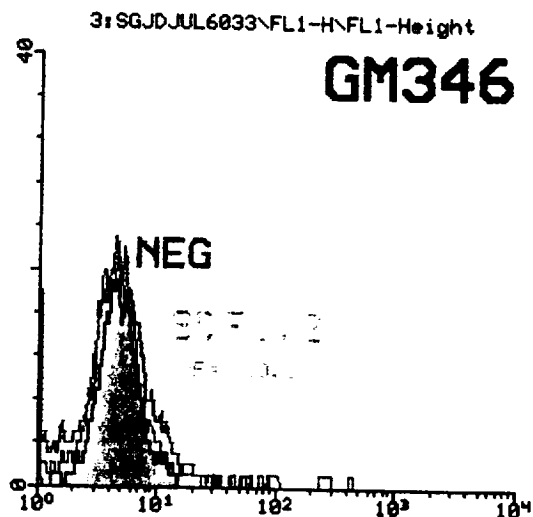
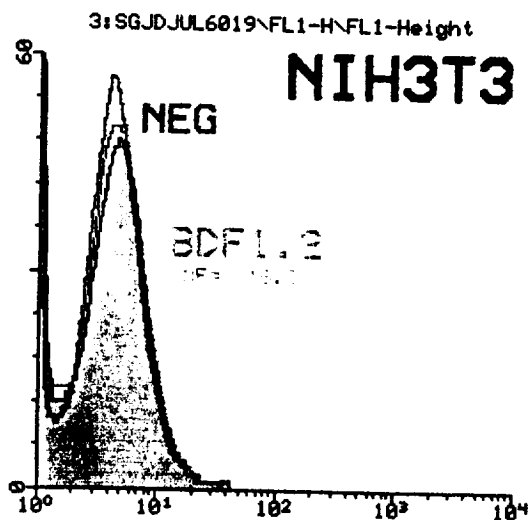
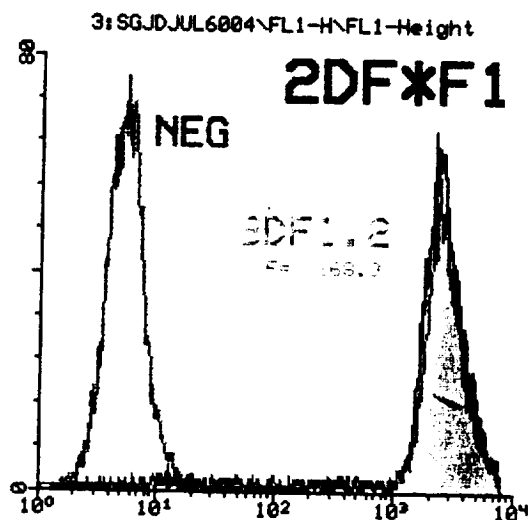
	ANTIBODY	NET FLUORESCENCE
HT1080	WSJ-2	+++ (253.30)
	WSJ-3	+/- (88.7)
	WSJ-4	+ (177.20)
	WSJ-5	++ (265.4)
	WSJ-6	+++ (339.3)
Hep3B	WSJ-2	-
	WSJ-3	-
	WSJ-4	-
	WSJ-5	-
	WSJ-6	-
CHO 77256	WSJ-2	- (47.8)
	WSJ-3	+ (115.9)
	WSJ-4	+ (119.1)
	WSJ-5	-
	WSJ-6	-
2DF*F1, B14I50, Mouse L, & FTO-2B	WSJ-2	-
	WSJ-3	-
	WSJ-4	-
	WSJ-5	-
	WSJ-6	-

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LYSYS II Version 1.0 11/90

DATE: 19-MAY-94

TIME: 5:01:46



NUMBER OF CELLS

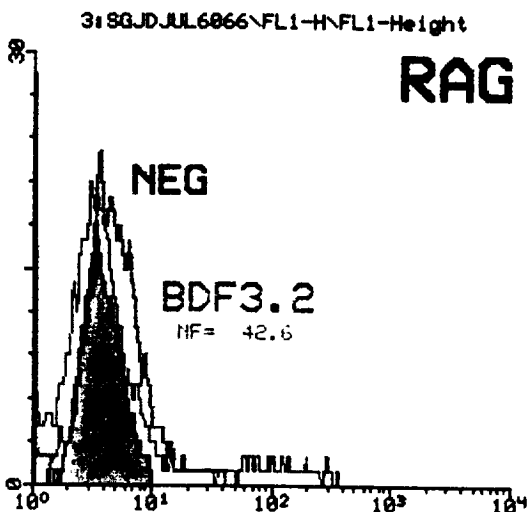
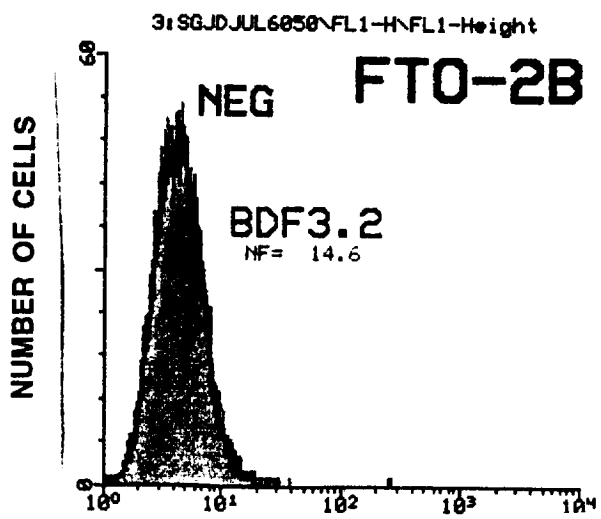
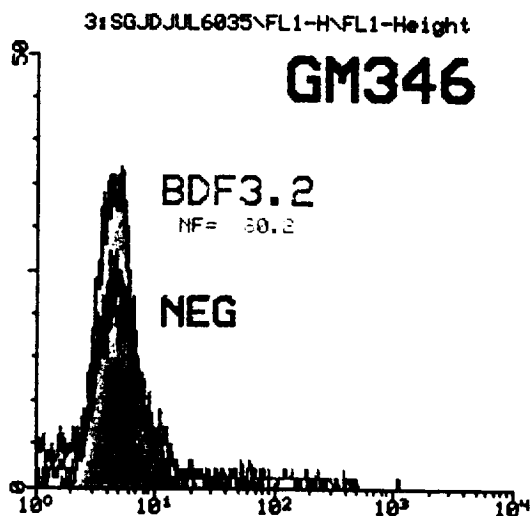
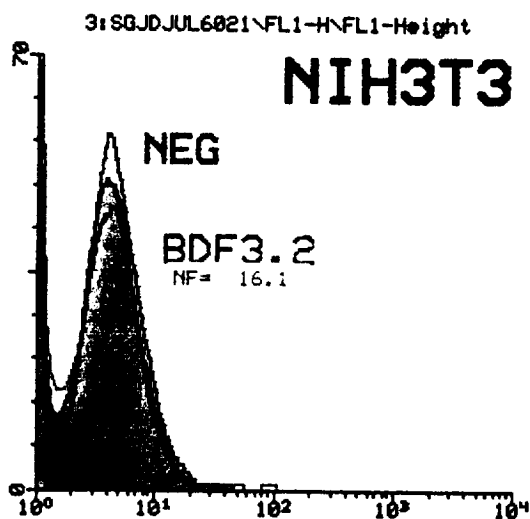
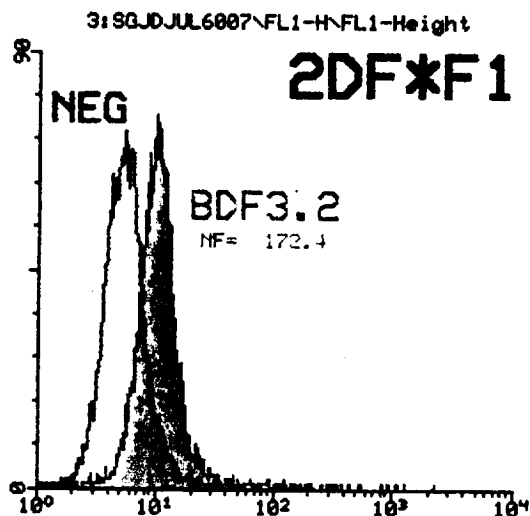
RELATIVE FLUORESCENCE INTENSITY

PECTON
DICKINSON

LYSYS II Version 1.0 11/90

DATE: 19-MAY-94

TIME: 2:38:56



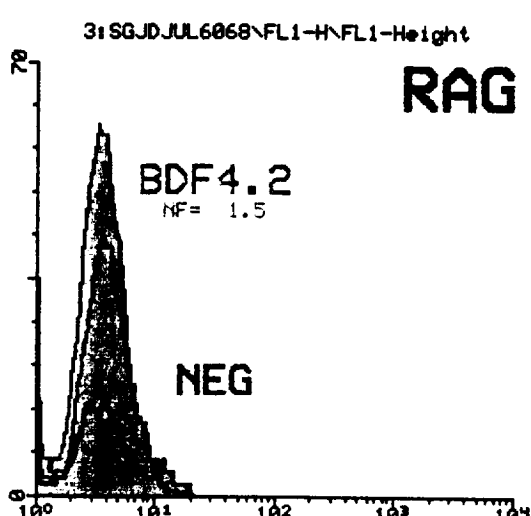
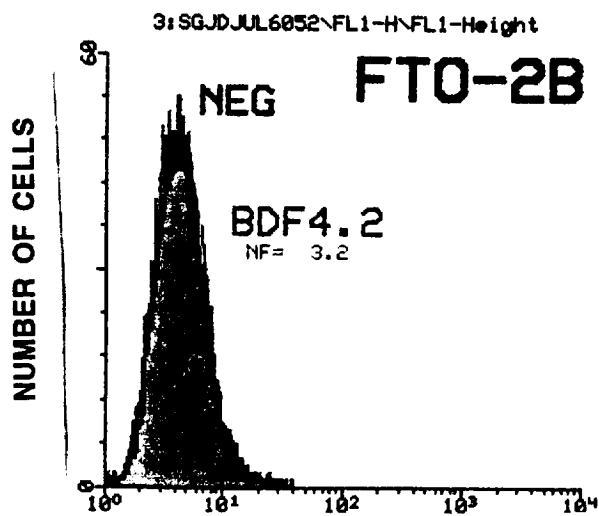
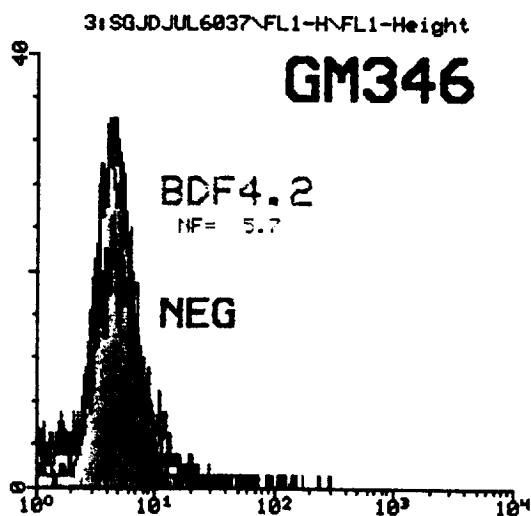
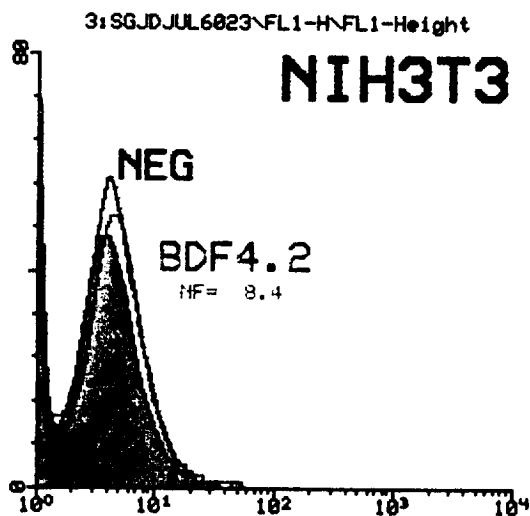
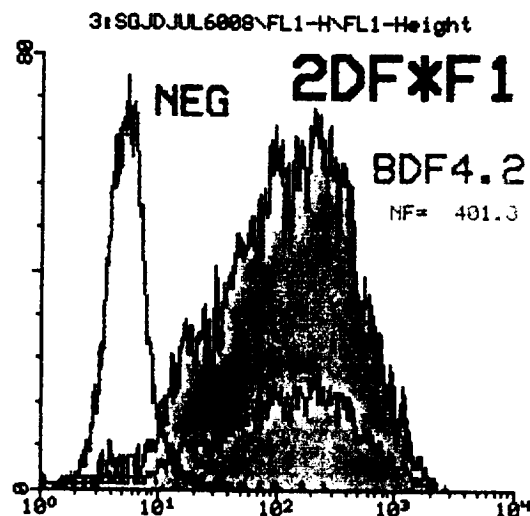
RELATIVE FLUORESCENCE INTENSITY

BECTON
DICKINSON

LYSYS II Version 1.0 11/90

DATE: 19-MAY-94

TIME: 2:58:22



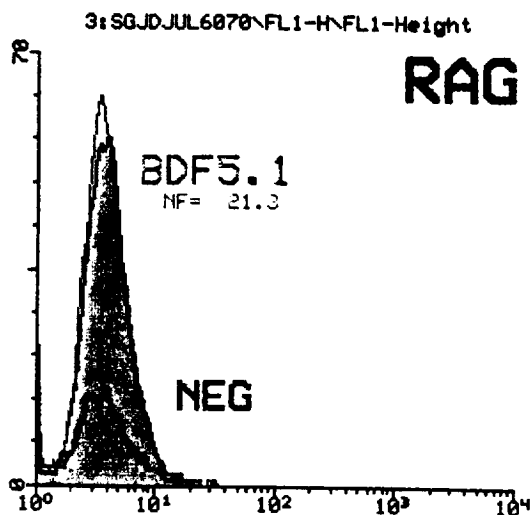
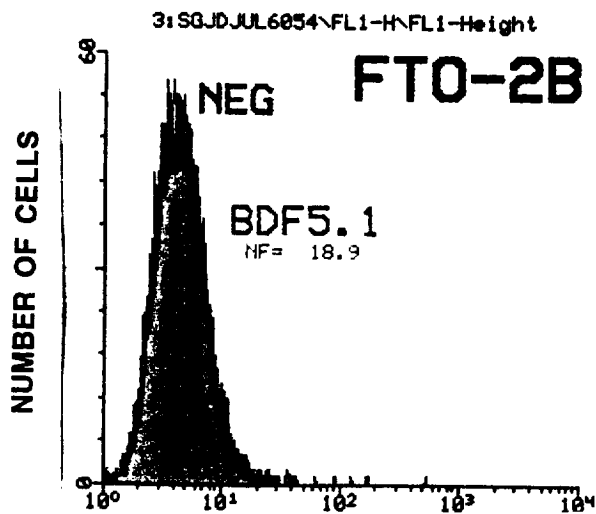
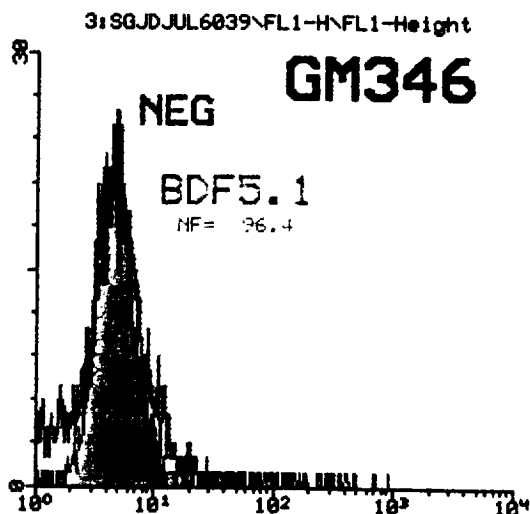
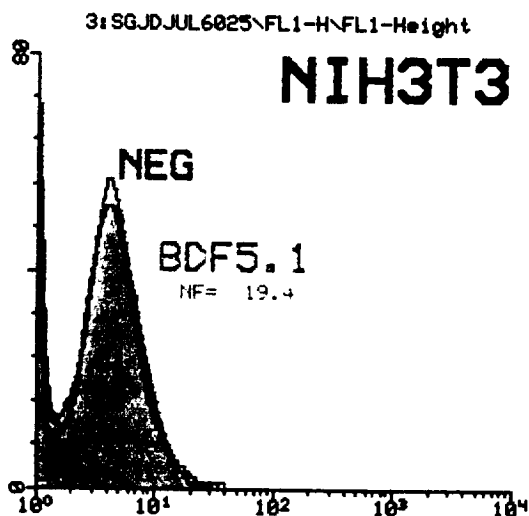
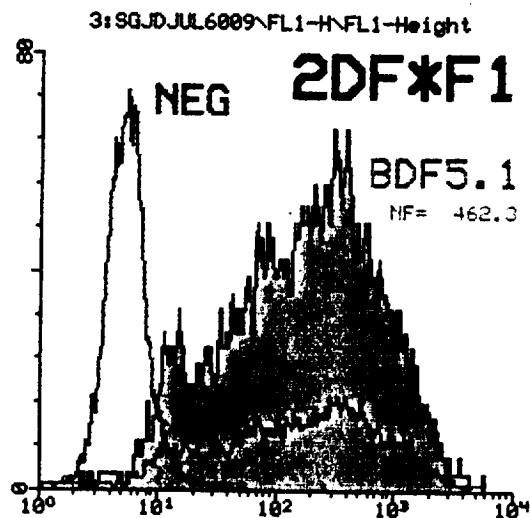
RELATIVE FLUORESCENCE INTENSITY

BECTON
DICKINSON

LYSYS II Version 1.0 11/90

DATE: 19-MAY-94

TIME: 3:15:26



NUMBER OF CELLS

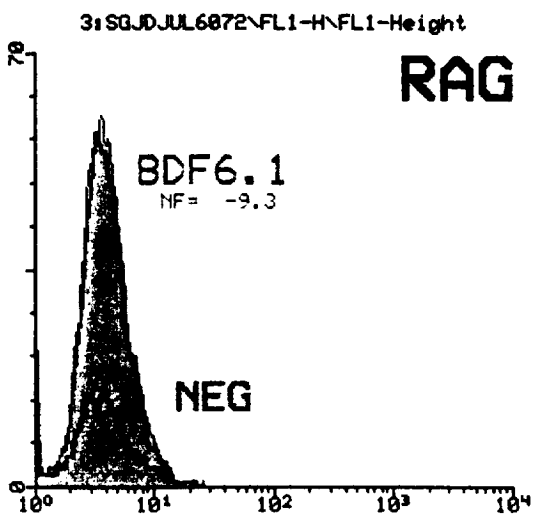
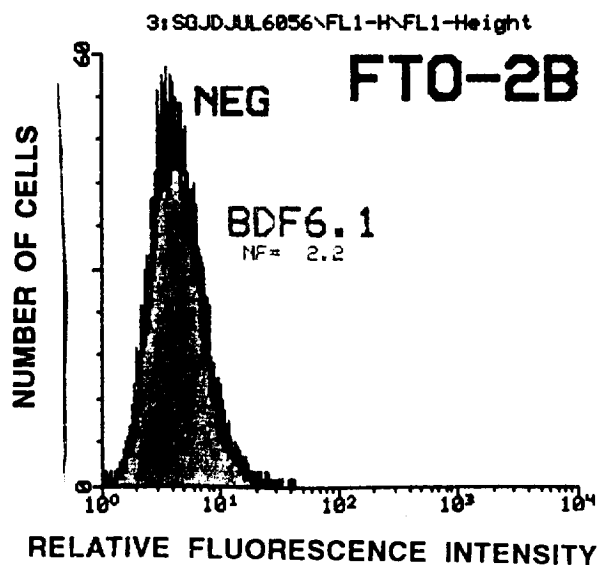
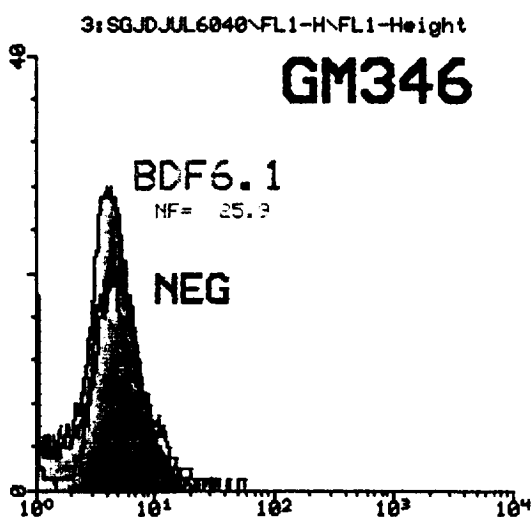
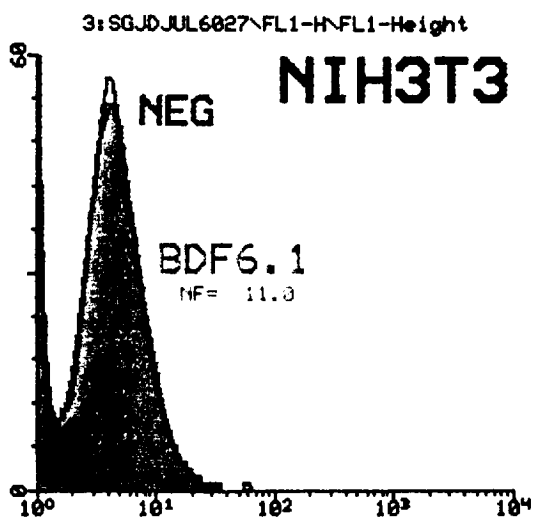
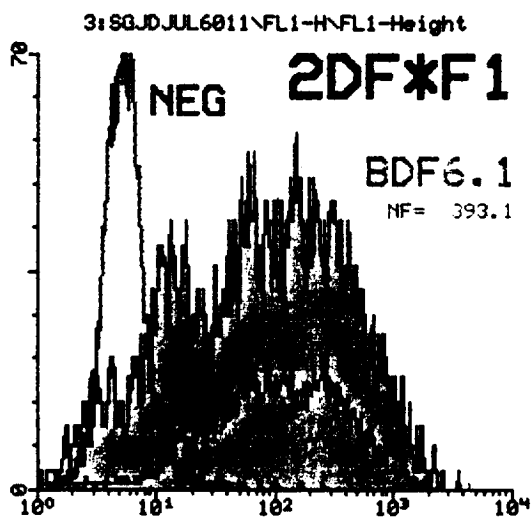
RELATIVE FLUORESCENCE INTENSITY

BECTON
DICKINSON

LYSYS II Version 1.0 11/90

DATE: 19-MAY-94

TIME: 3:32:54



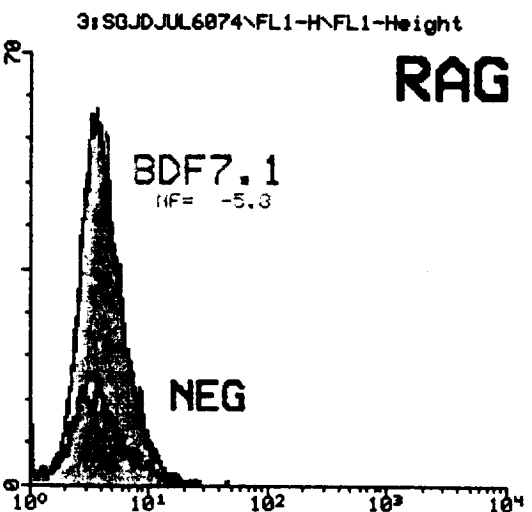
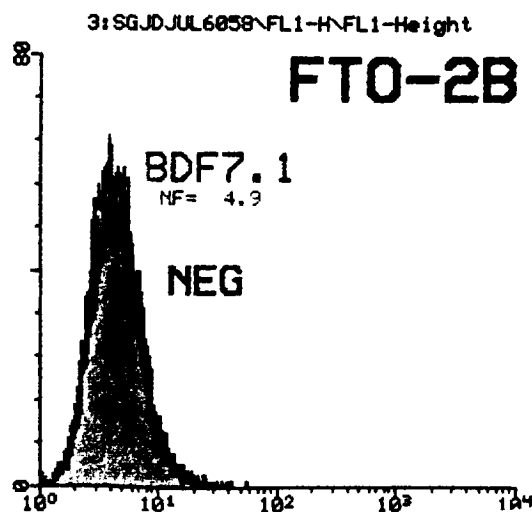
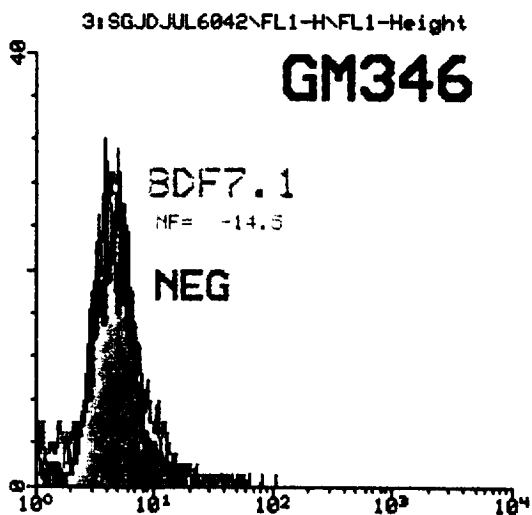
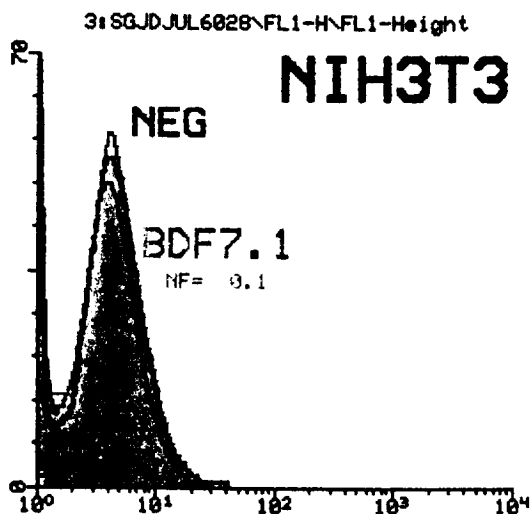
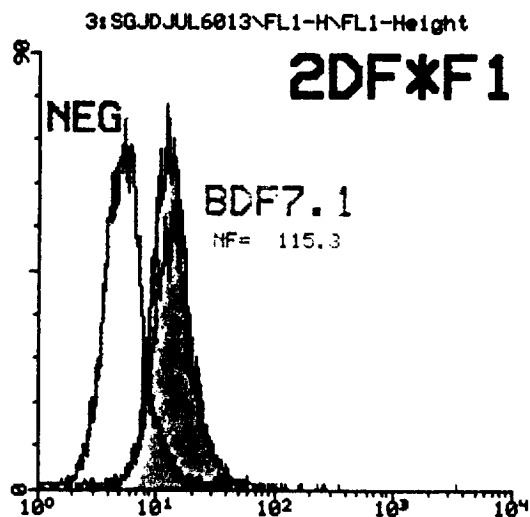
BECTON

DICKINSON

LYSYS II Version 1.0 11/90

DATE: 19-MAY-94

TIME: 4:32:46



NUMBER OF CELLS

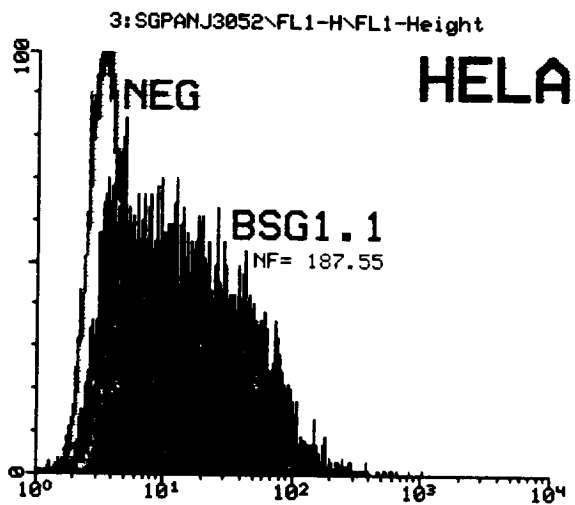
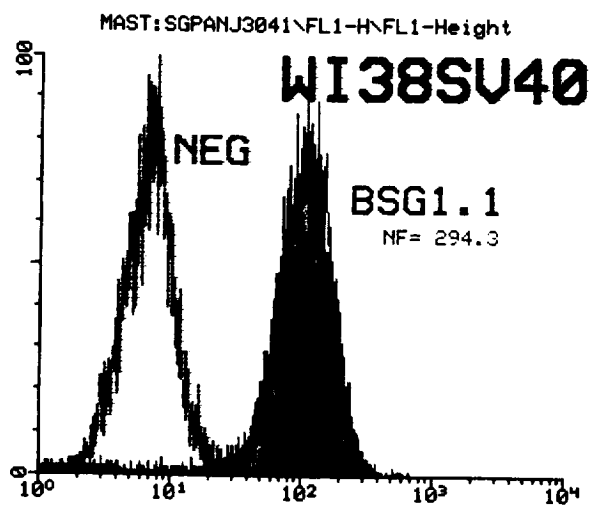
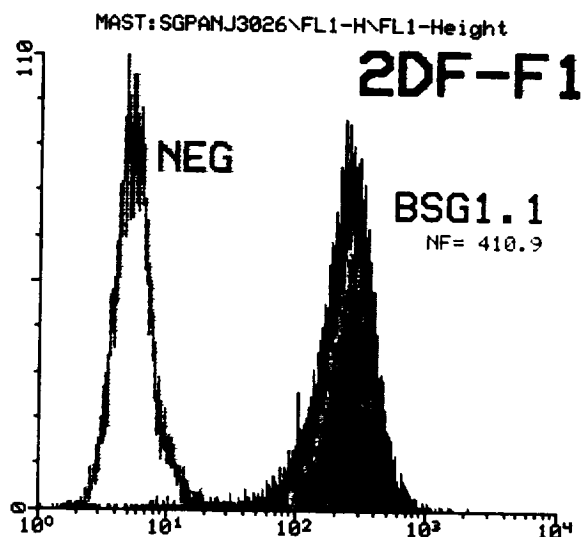
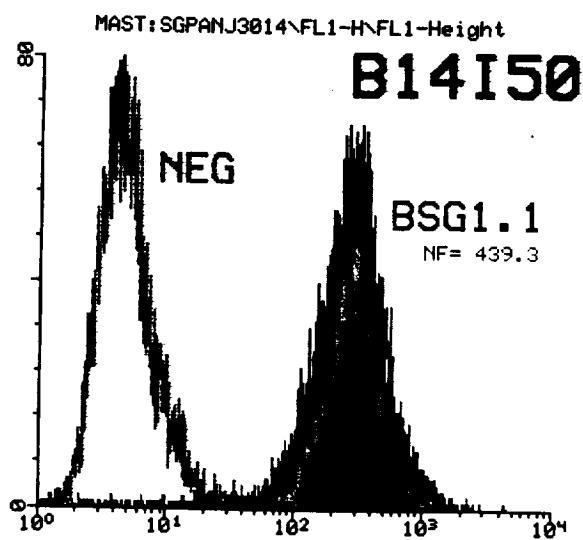
RELATIVE FLUORESCENCE INTENSITY

BECTON
DICKINSON

LYSYS II Version 1.0 11/90

DATE: 13-JAN-94

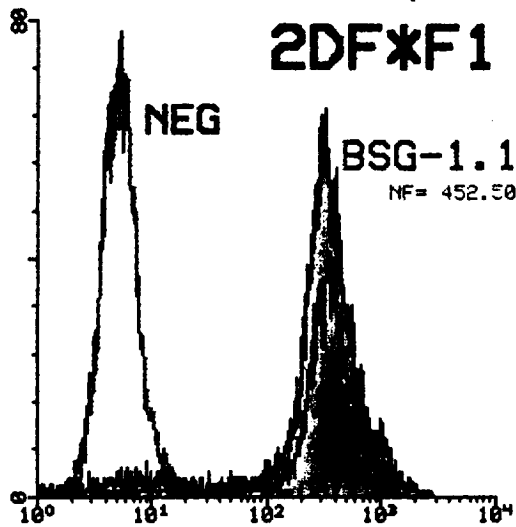
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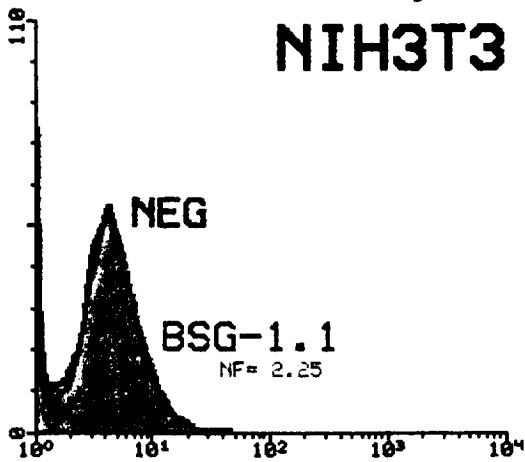
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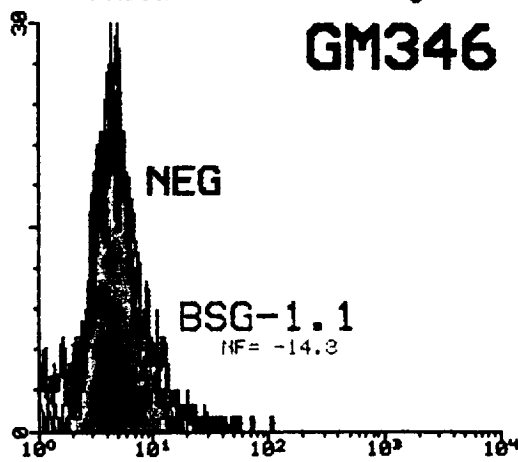
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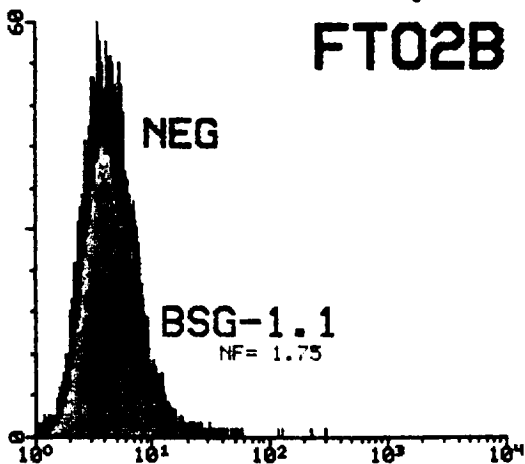
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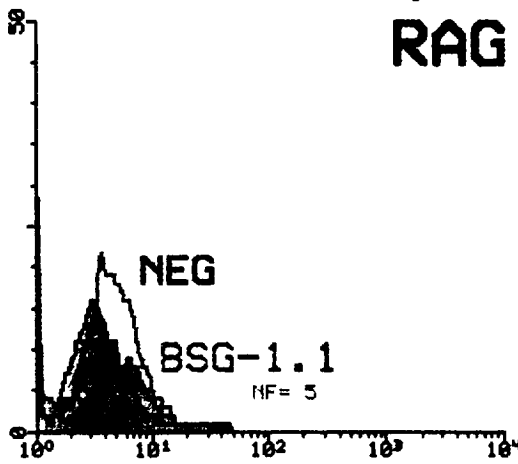
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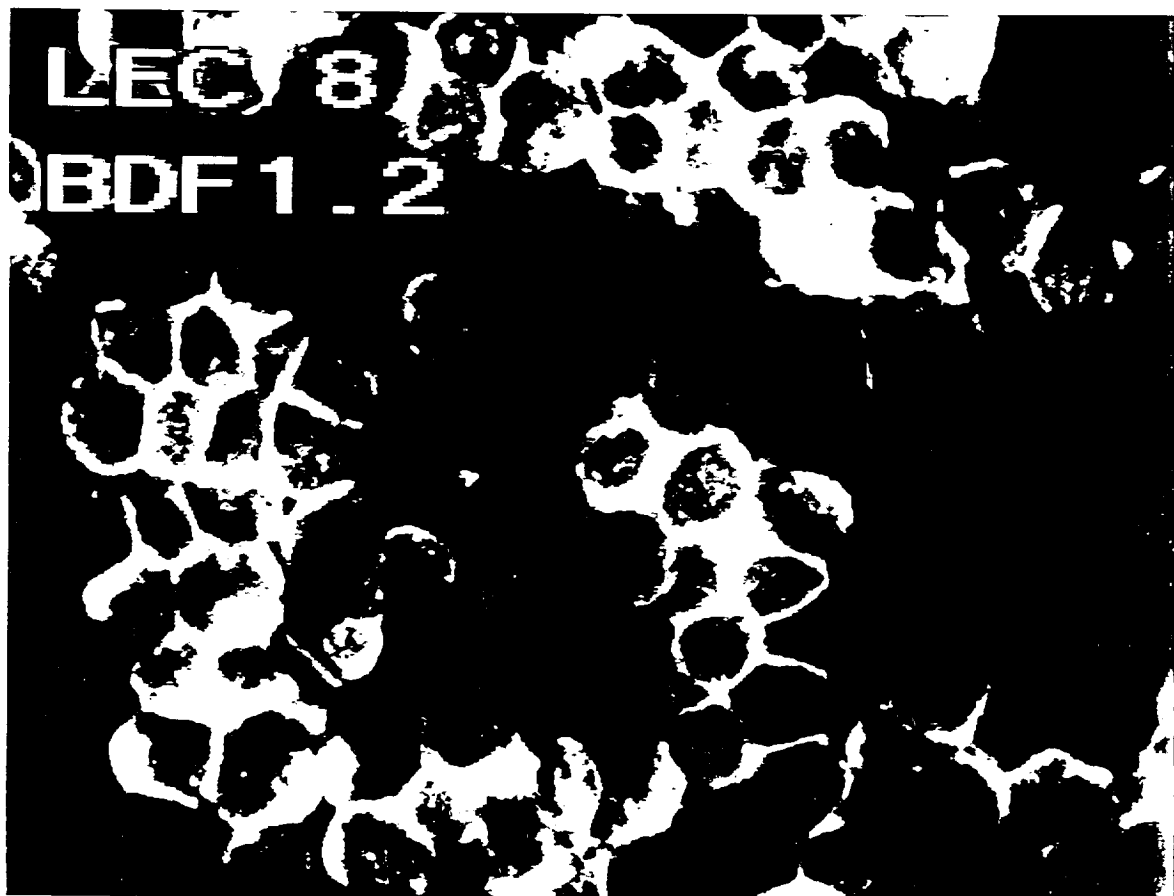


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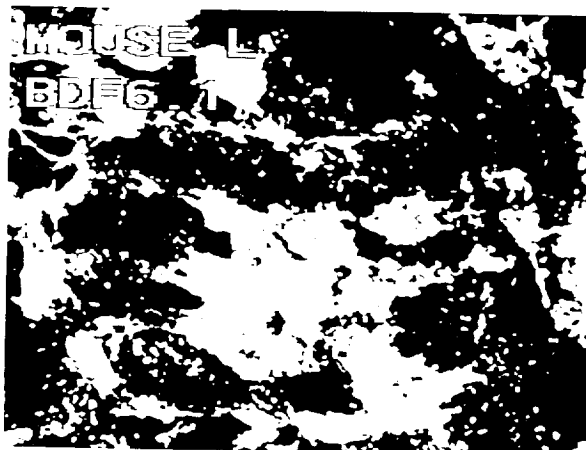
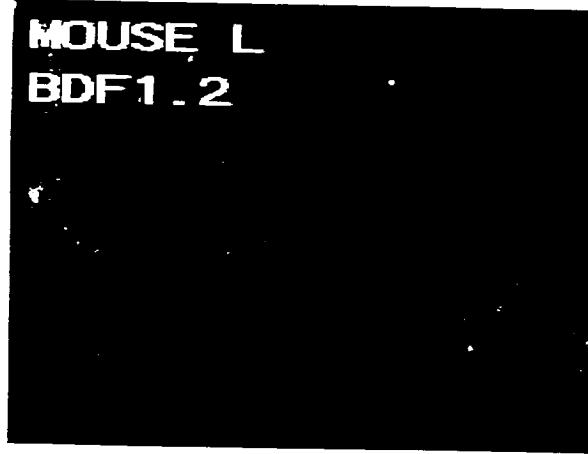
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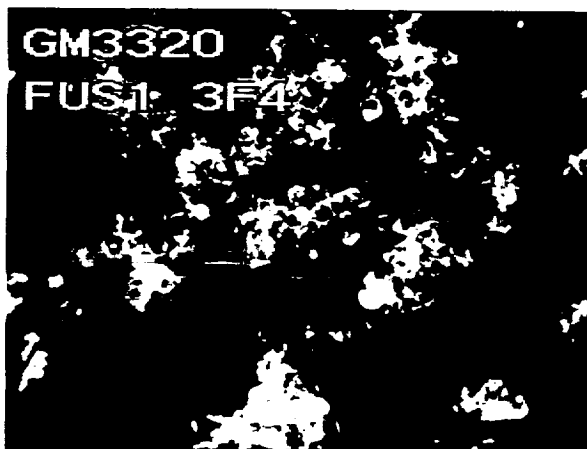
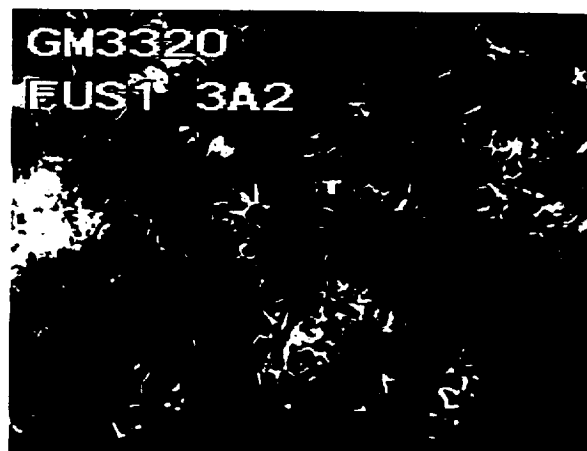


BDF 1.2 on Acetone Fixed Lec 8 and CHO Pro-5 cells

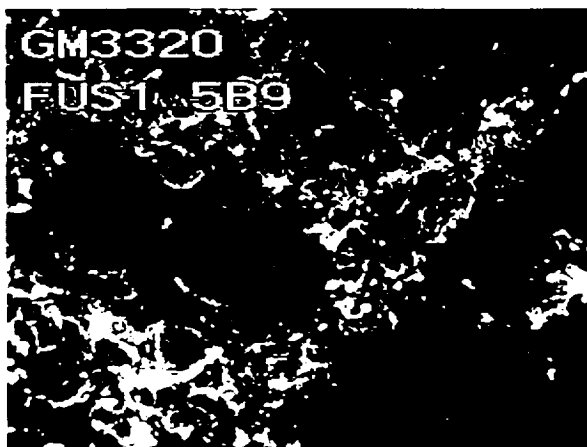
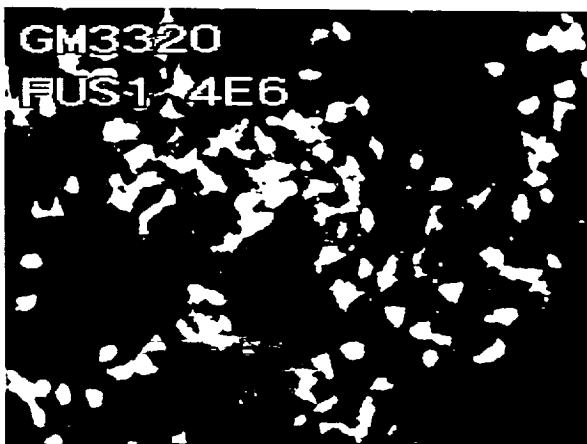
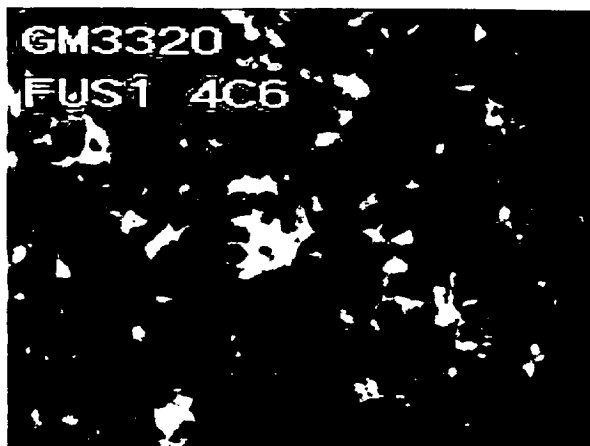




Acetone Fixed Mouse L cells



*VARIOUS BINDING PATTERNS FROM GM3320
FUSION 1 ON GM3320 ACETONE FIXED SLIDES*



GM3320 FUS 1
PLATE 2B6

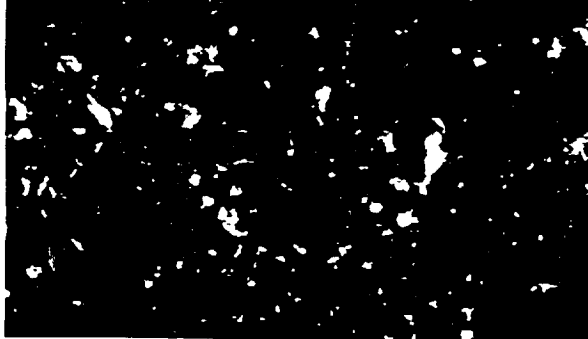


PLATE 1H12

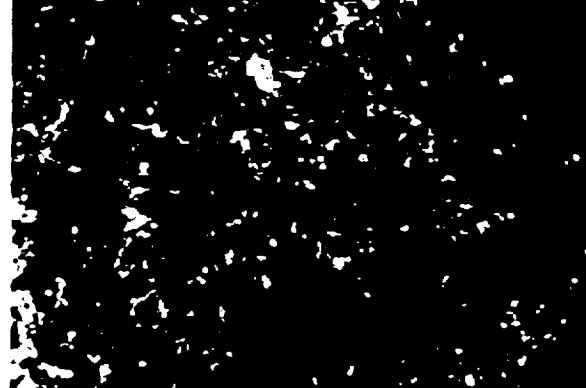
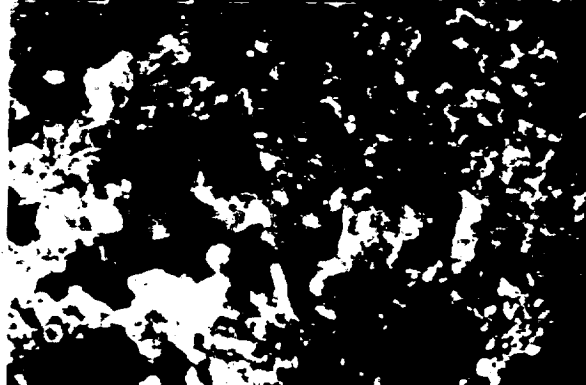


PLATE 2B5



PLATE 2E12



VARIOUS BINDING PATTERNS FROM GM3320
FUSION 1 ON GM3320 ACETONE FIXED SLIDES

PLATE 2B6

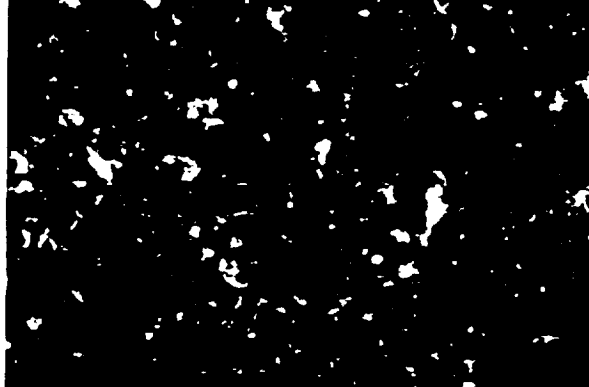


PLATE 1H12

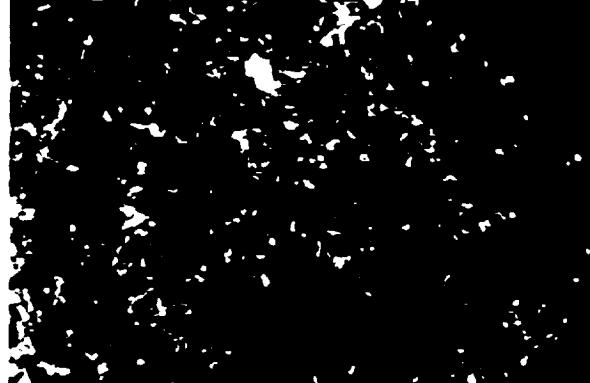


PLATE 2B5



PLATE 2E12



GM3320 FUS 1
PLATE 3A2

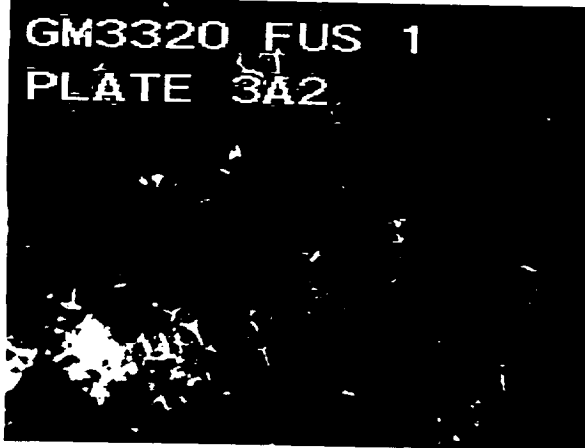


PLATE 3E2

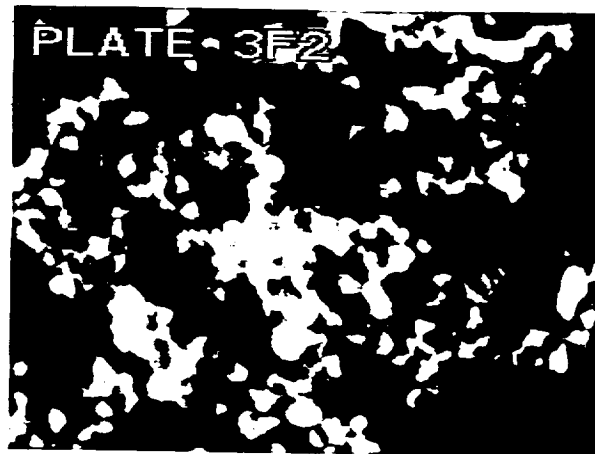


PLATE 3F4

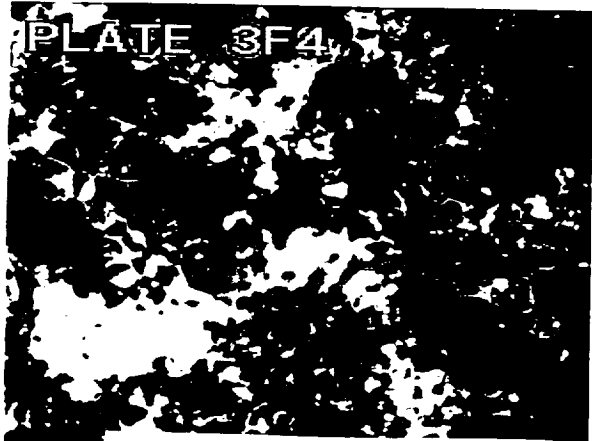
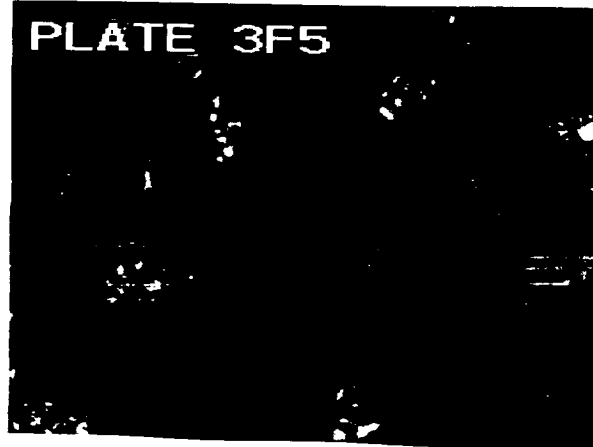
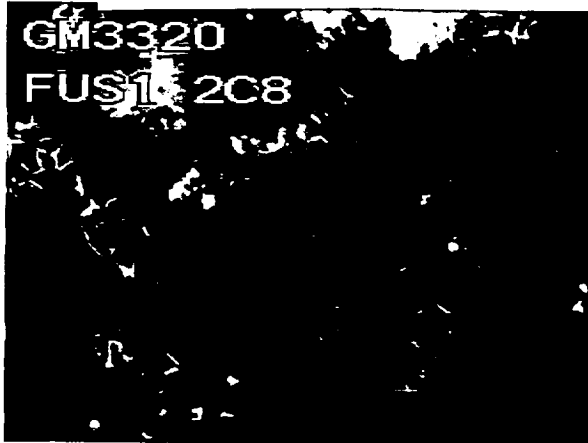


PLATE 3F5

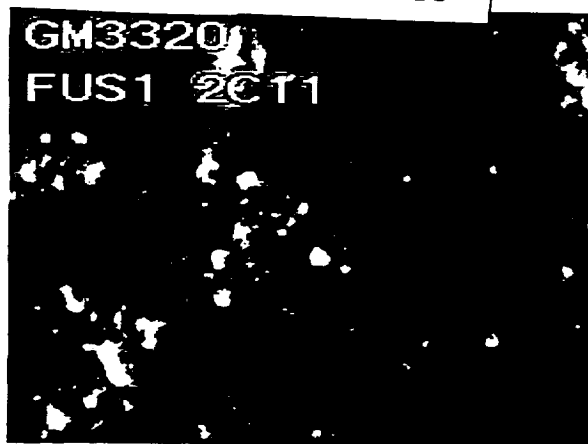


*VARIOUS BINDING PATTERNS FROM GM3320
FUSION 1 ON GM3320 ACETONE FIXED SLIDES*

GM3320
FUS1 2C8



GM3320
FUS1 2C11

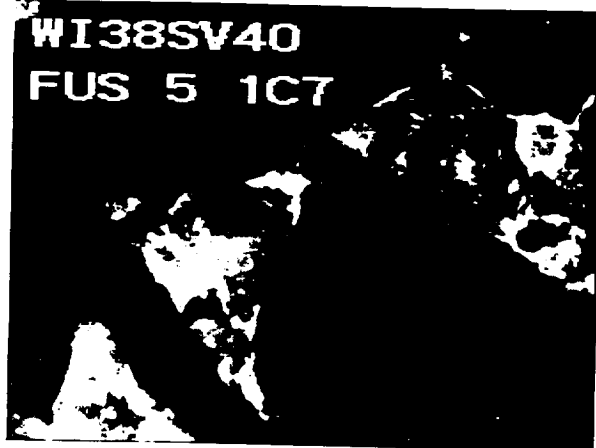
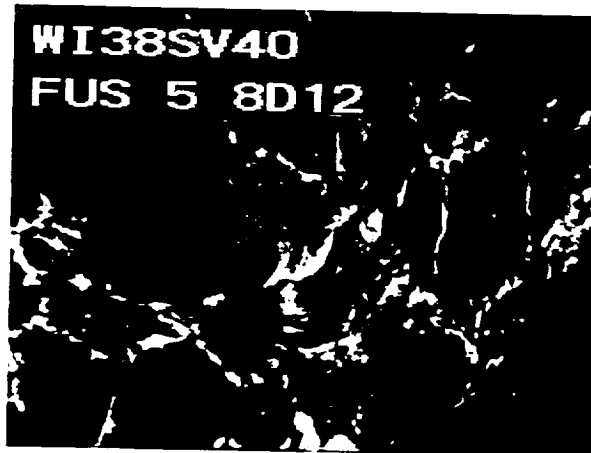
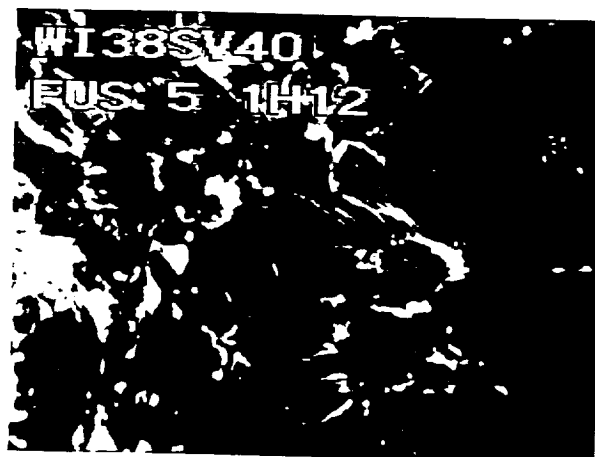


GM3320
FUS1 2D11

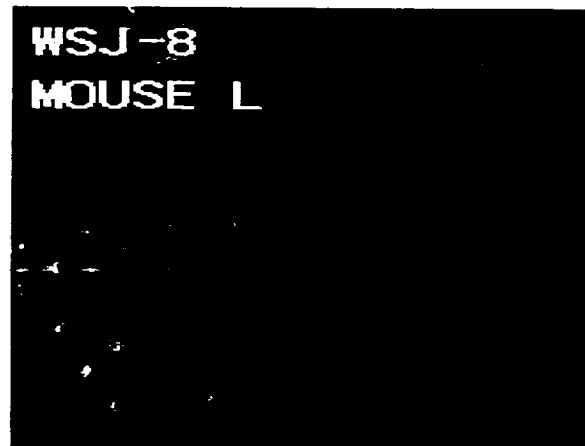
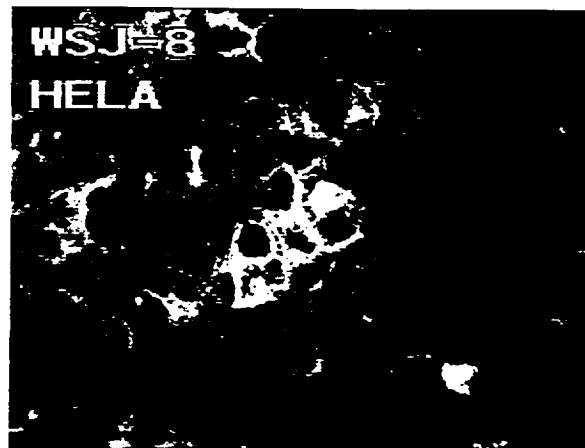
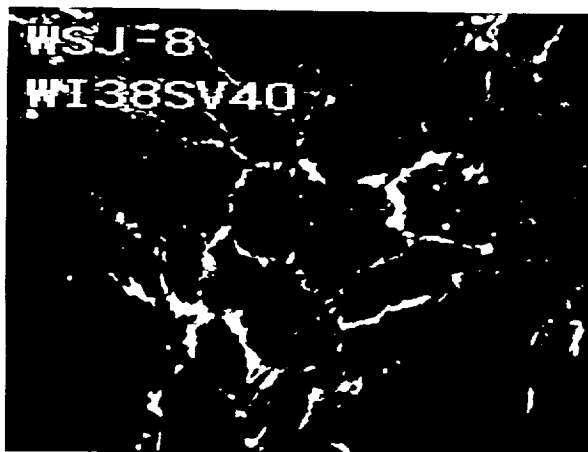


GM3320
FUS1 2E12

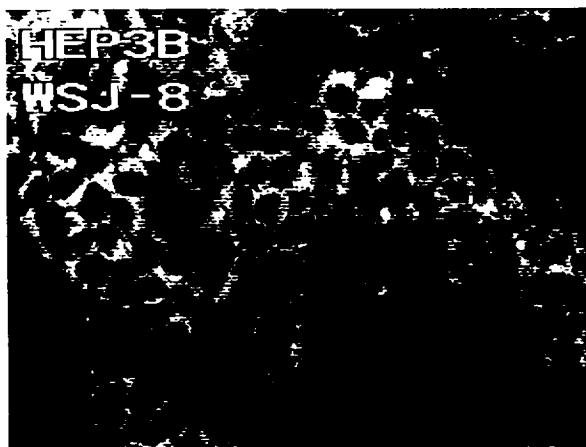
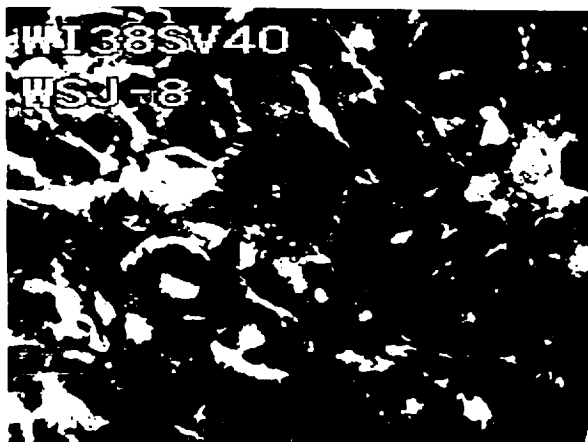


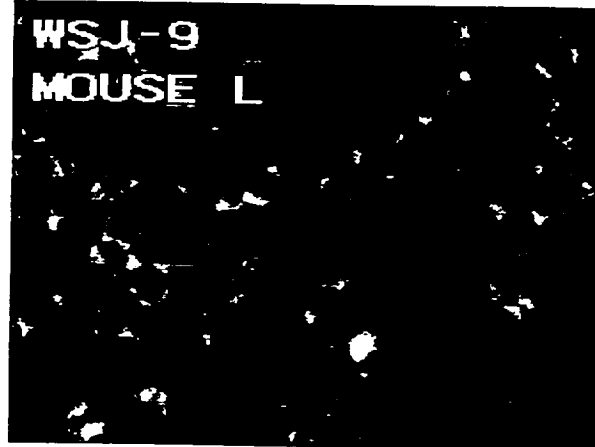
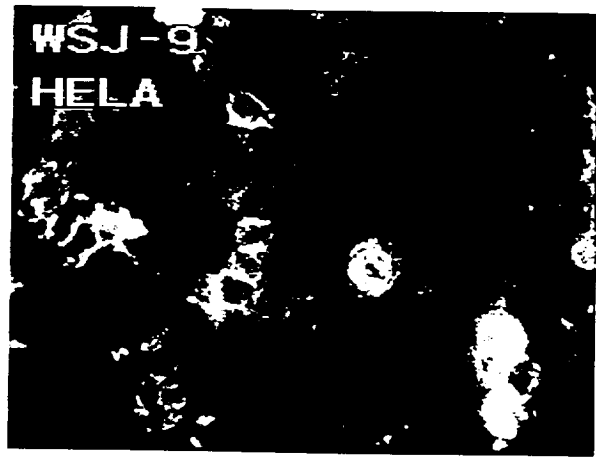
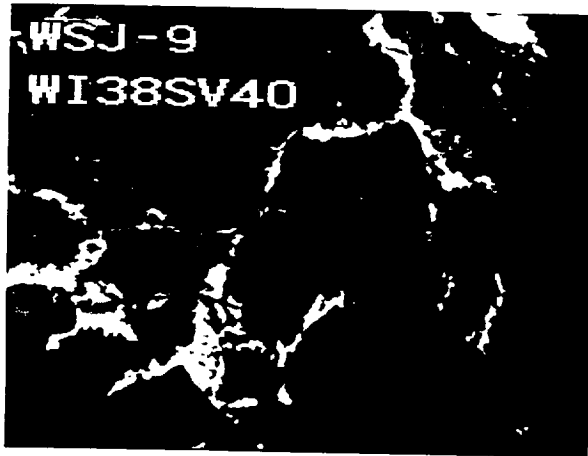


ANTIBODIES CURRENTLY BEING CLONED;
WI38sv40 ACETONE FIXED SLIDES

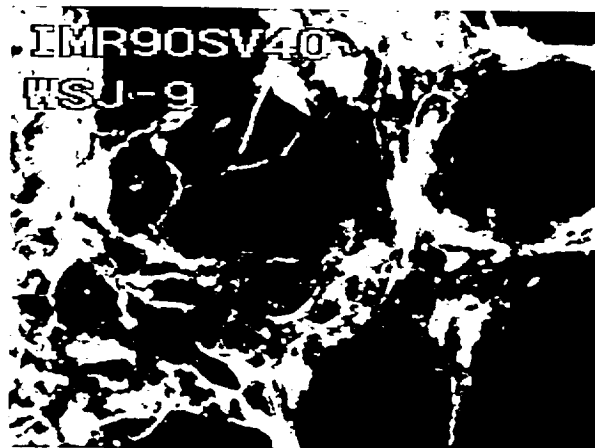
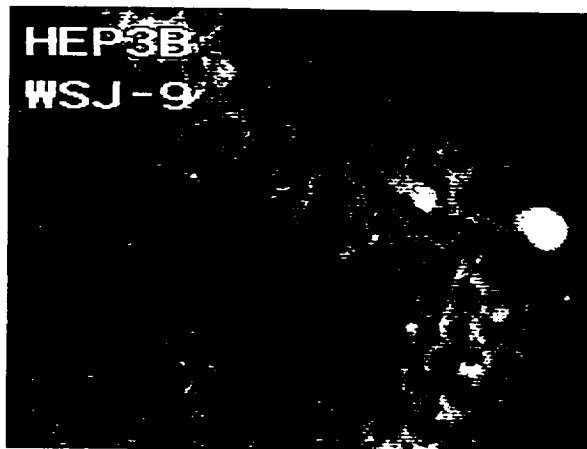
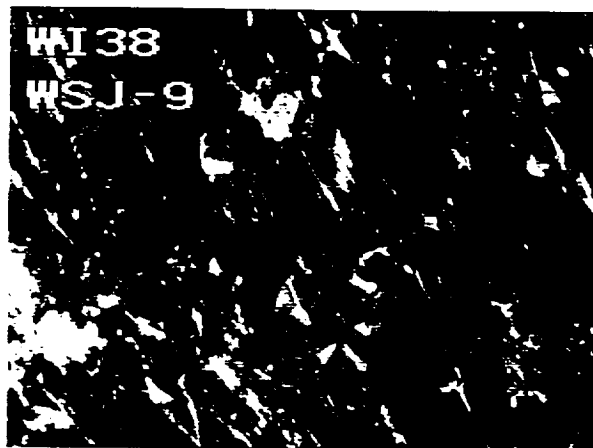
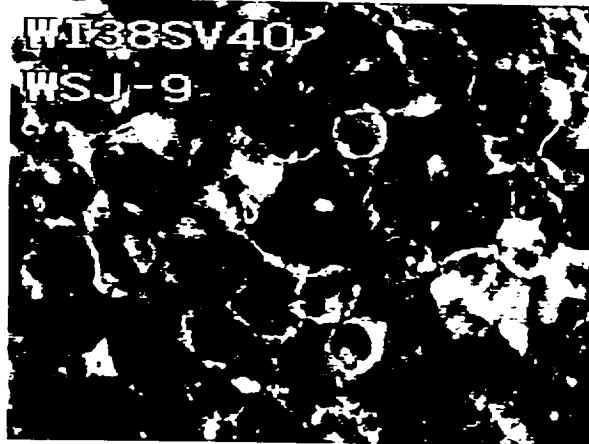


ACETONE FIXED SLIDES



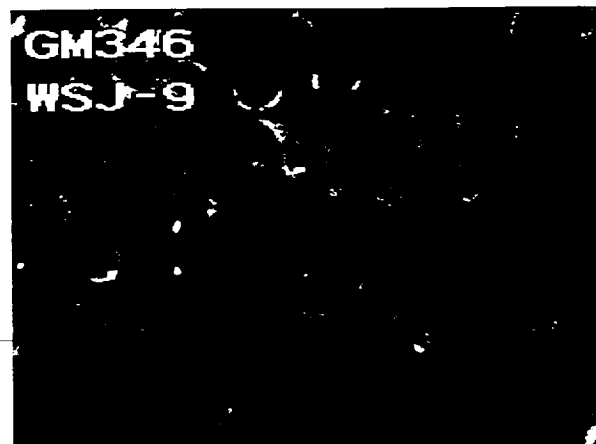


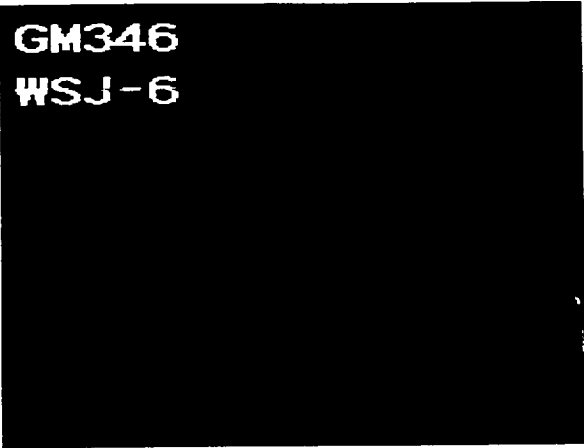
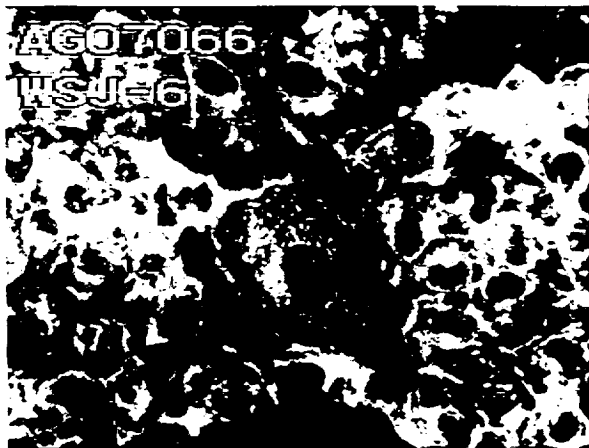
ACETONE FIXED SLIDES



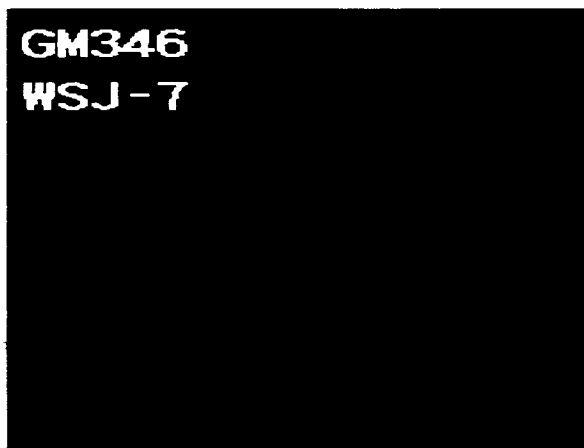
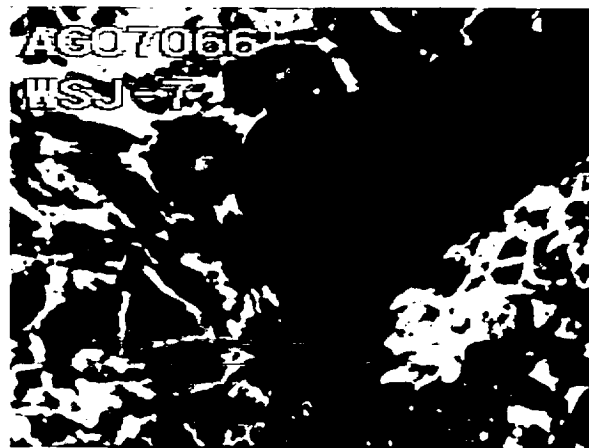


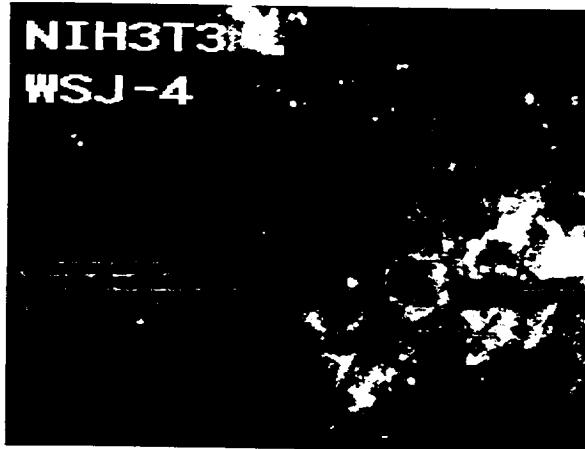
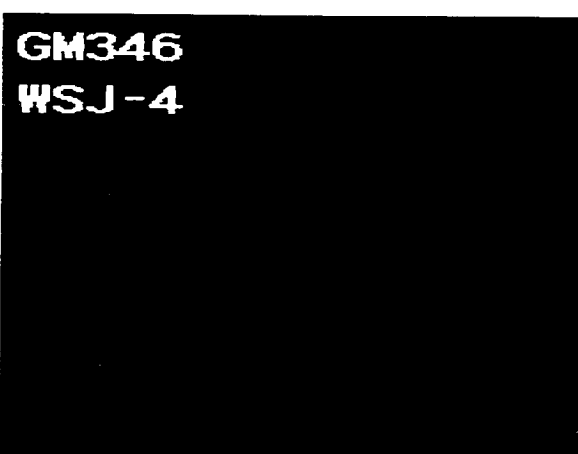
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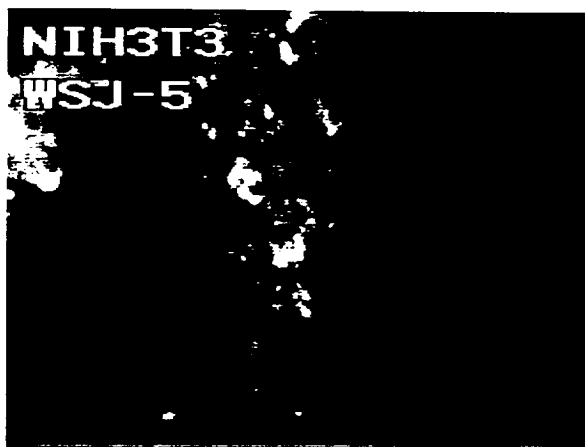
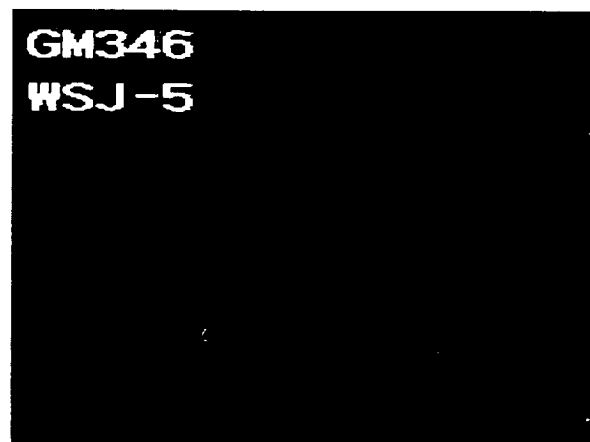


ACETONE FIXED SLIDES



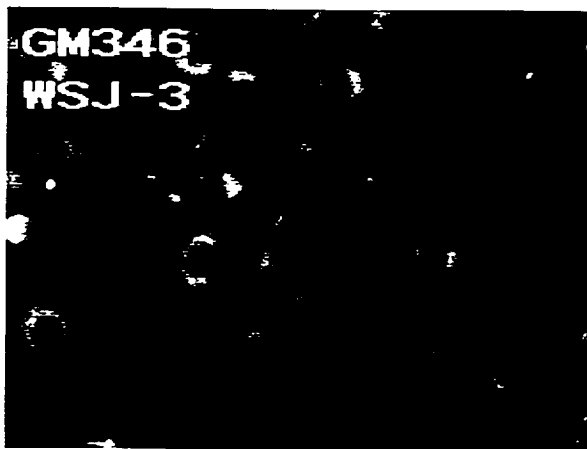


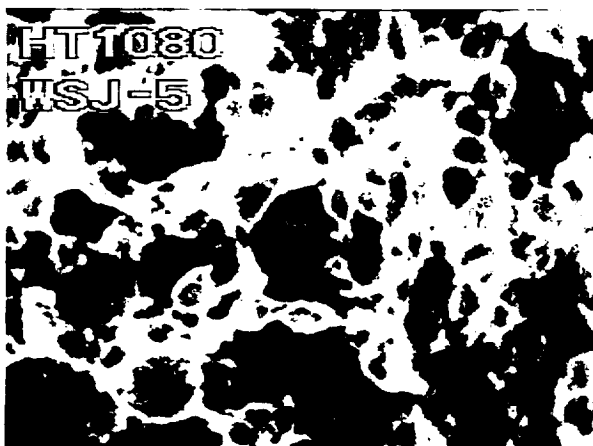
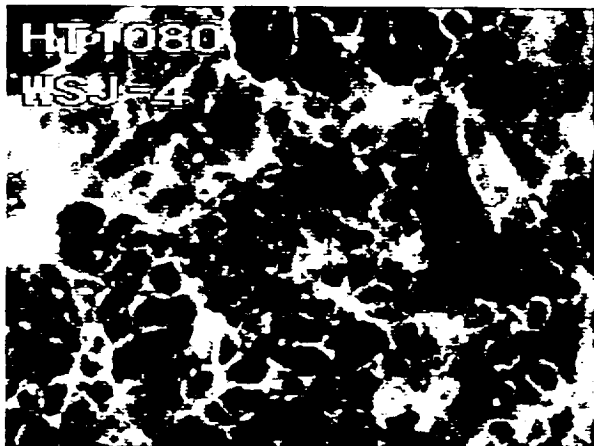
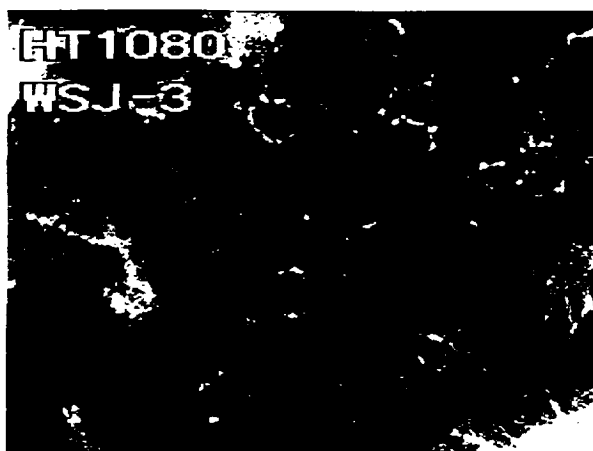
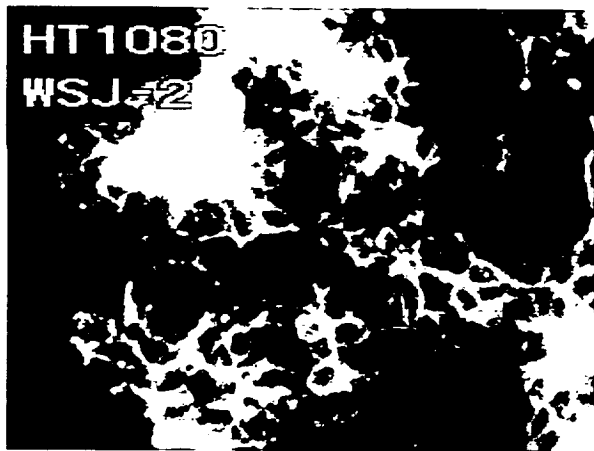
ACETONE FIXED SLIDES





ACETONE FIXED SLIDES





LIVE CELLS

